FIG 1

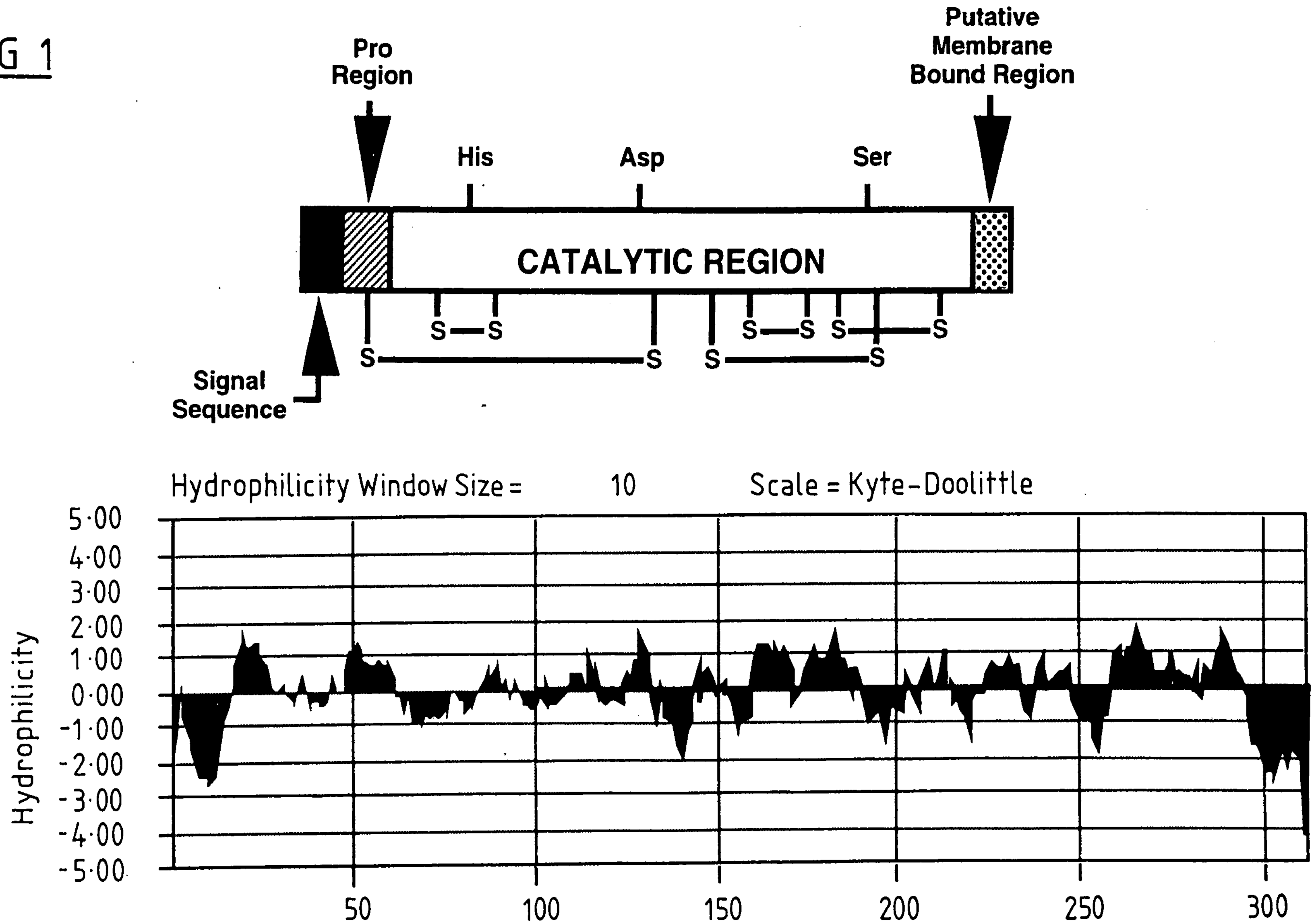

Pro
Region
Putative
Membrane
Bound Region
His
Asp
Ser
CATALYTIC REGION
S—S
S—S
S—S
S—S
S—S
S—S
Signal
Sequence
Hydrophilicity Window Size = 10
Scale = Kyte-Doolittle
Hydrophilicity
5·00
4·00
3·00
2·00
1·00
0·00
-1·00
-2·00
-3·00
-4·00
-5·00
50
100
150
200
250
300

FIG 2A

FIG 2A(I)	FIG 2A(II)

Sequence comparison of HELA2(Testisin) and prostasin

```
                  Signal sequence                Light Chain
prostasin  MAQKGVLGPGQLGAVAILLYLGLLRSGTGAEGA--EAPCG-VAPQARITGGSSAVA
HELA2      MGARGAL----L--LALLLARAGLRKPESQEAAPLSGPCGRRVITSRIVGGEDAEL
           *  .* *   *  .*.**   **   * *  ***     .** **  *
                                             S-S
             ASP                                       N-gly
              *
prostasin  KVSTLKDIIPHPSYLQEGSQGDIALLQLSRPITFSRYIRPICLPAANASFPNGLHC
HELA2      TRYFVSNIYLSPRYLG-NSPYDIALVKLSAPVTYTKHIQPICLQASTFEFENRTDC
             .  *  * **  .*  ****..** *.*...  *.**** *..  * *  *
                                                          N-gly
                                  S-S
   SER
    *
prostasin  ACQGDSGGPLSCPVEGLWYLTGIVSWGDACGARNRPGVYTLASSYASWIQSKVTEL
HELA2      ACFGDSGGPLACNKDGLWYQIGVVSWGVGCGRPNRPGVYTNISHHFEWIQ------
           ** *******.*  .****  *.****  **  *******  *    ***
                       S-S                     N-gly
```

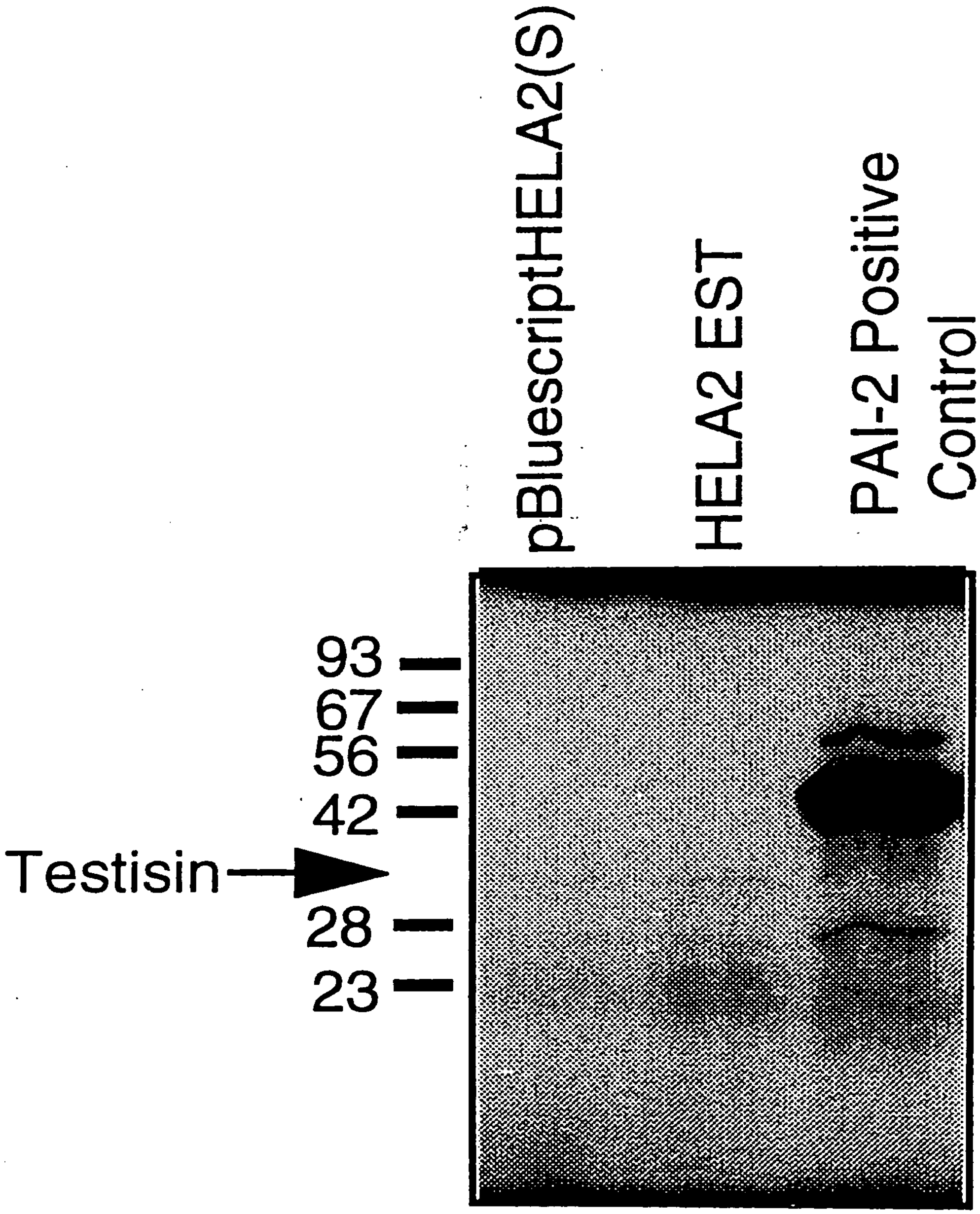
FIG 2A(I)

Sequence comparison of HELA2(Testisin) and prostasin

```
                                            HIS
                                             *
prostasin  GQWPWQVSITYEGVHVCGGSLVSEQWVLSAAHCFPSEHHK-EAYEVKLG----AHQLDSYSEDA
HELA2      GRWPWQGSLRLWDSHVCGVSLLSHRWALTAAHCFETDLSDPSGWMVQFGQLTSMPSFWSLQAYY
           *.**** *.      **** **.* .* *.*****  ..        *. *         *
                          |______S-S______|    YS (Long Isoform)

                       Heavy Chain
prostasin  TVTGWGHVAPSVSLLTPKPLQQLEVPLISRETCNCLYNIDAKPEEPHFVQEDMVCAGYVEGGKD
HELA2      WVTGWGYIKEDEALPSPHTLQEVQVAIINNSMCNHLFLKYSFRKD--IFG-DMVCAGNAQGGKD
            *****  .   .*  .*. **...* .*  ** *.   .   .      ******   .****
                                     N-gly
                                    |__________S-S__________|

                                                    Putative
                                                    Transmembrane
                                                    Domain
prostasin  QPRVVPQTQESQPDSNLCGSHLAFSSAPAQGLLR   PILFLPLGLALGLLSPWL   SEH
HELA2      --KLMAQSGMSQPD-------------PS----W   PLLFFPLLWALPLLGPV
             ... *.  ****              *.       *.** **  ** ** *
```

FIG 2A(II)

FIG 2B



In vitro transcription / translation of HELA2 (Testisin)

FIG 3

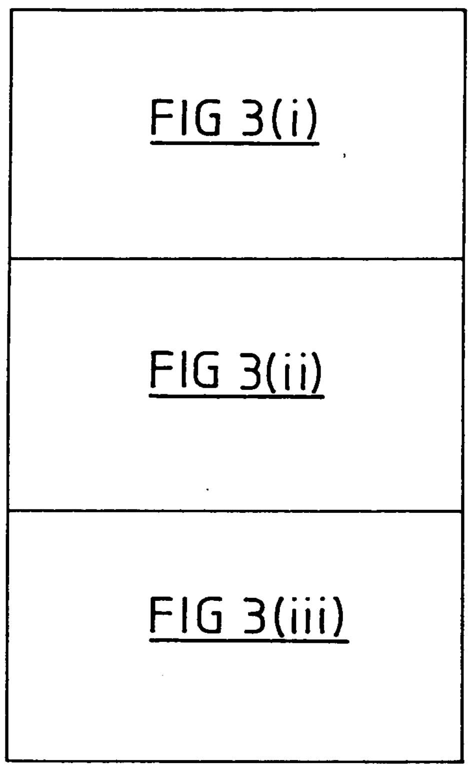
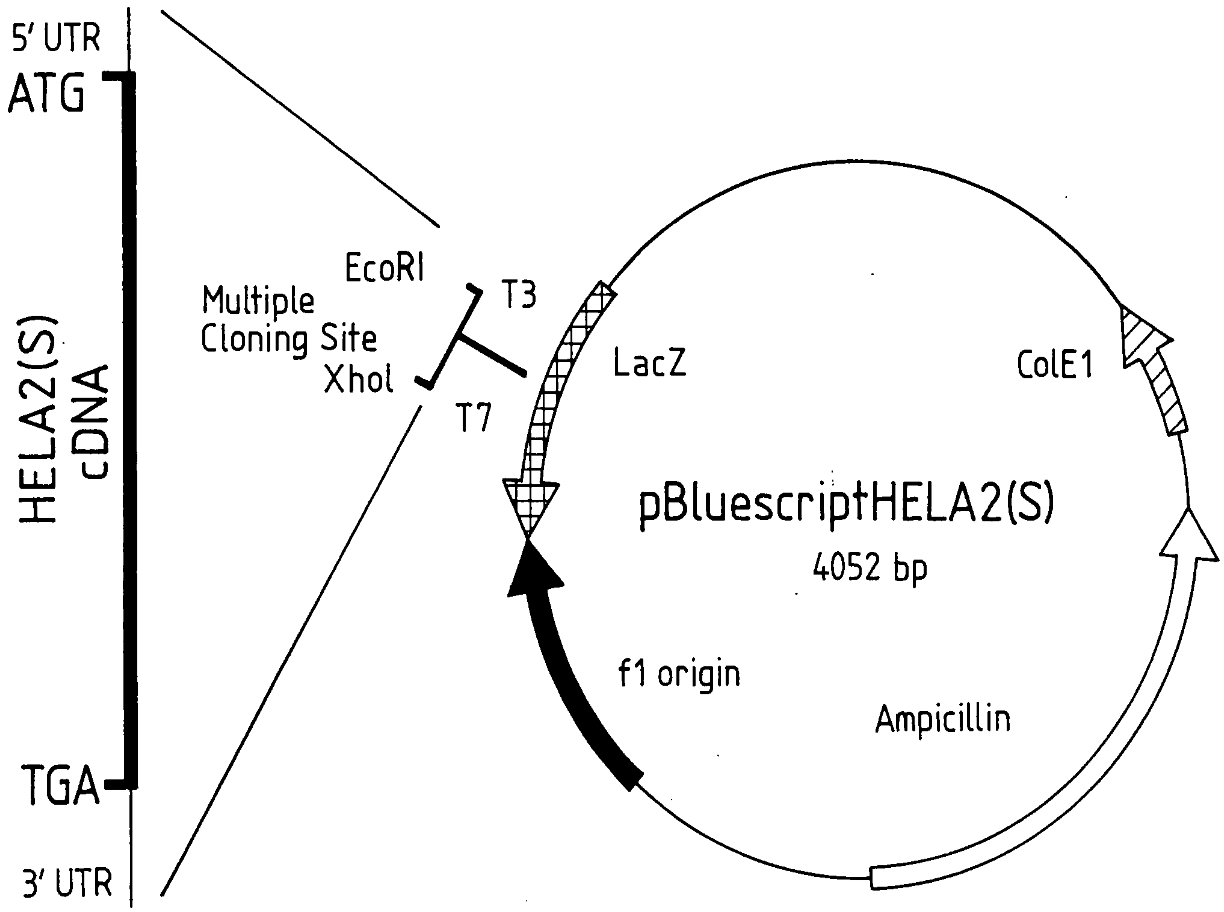
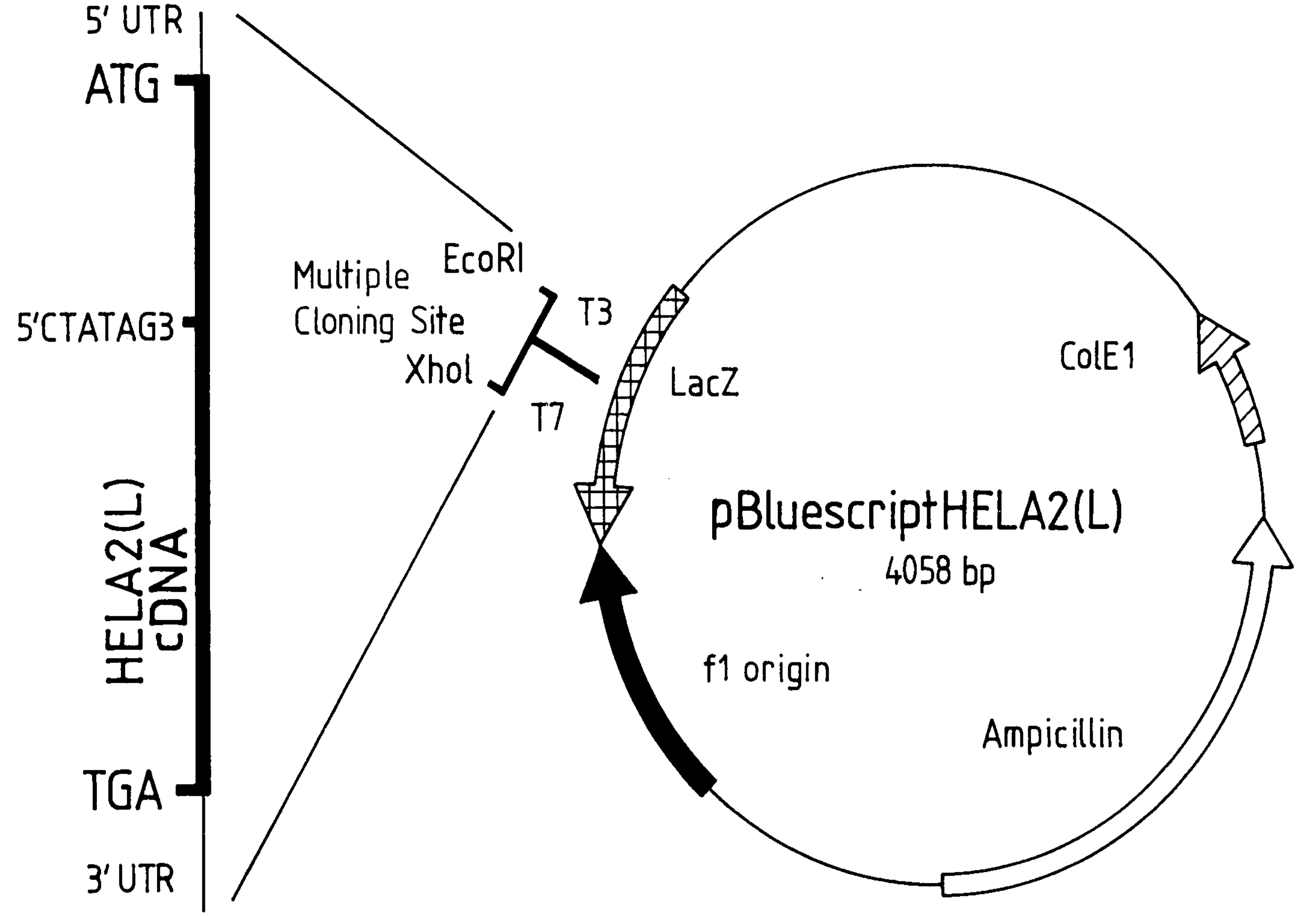


FIG 3(i)



HELA2 (Testisin) Short Isoform

FIG 3(ii)



HELA2 (Testisin) Long Isoform

HELA2 (Testisin) Restriction Enzyme Map

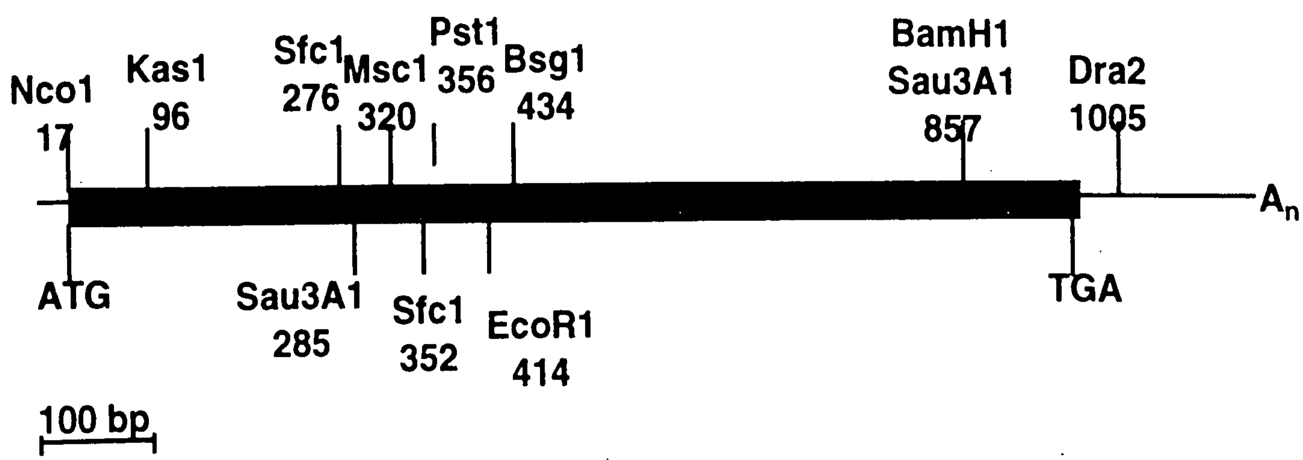
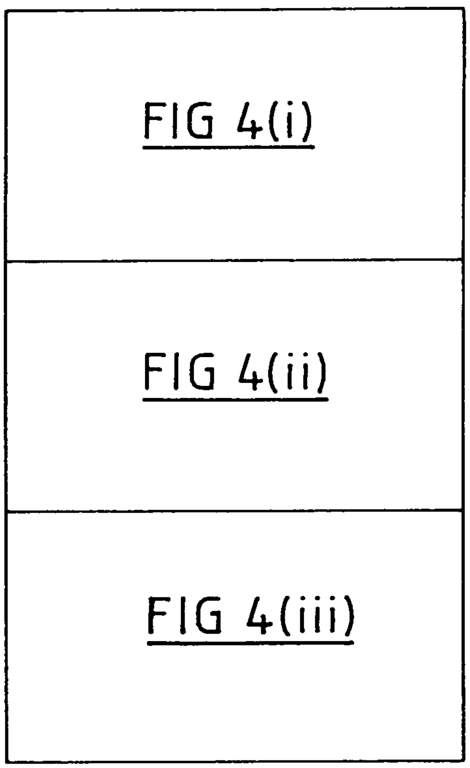


FIG 3(iii)

FIG 4



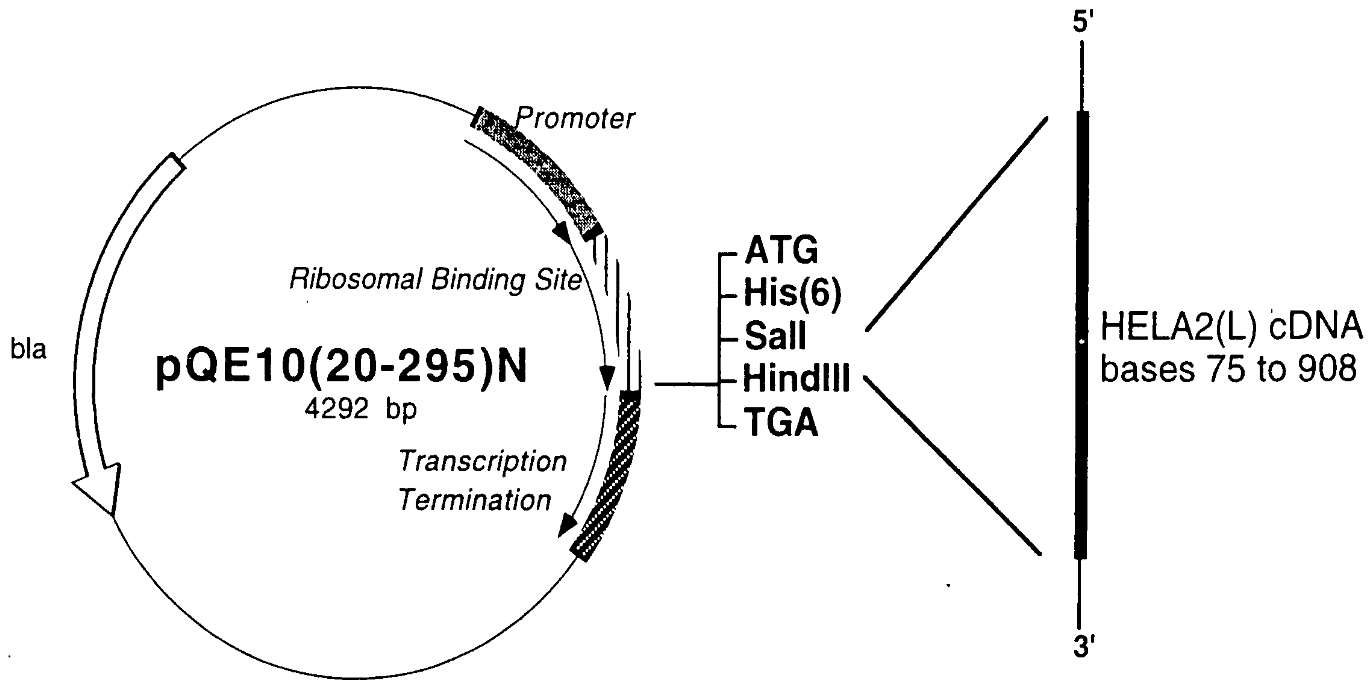

Promoter
Ribosomal Binding Site
bla
pQE10(20-295)N
4292 bp
Transcription
Termination
ATG
His(6)
SalI
HindIII
TGA
5'
3'
HELA2(L) cDNA
bases 75 to 908

FIG 4(i)

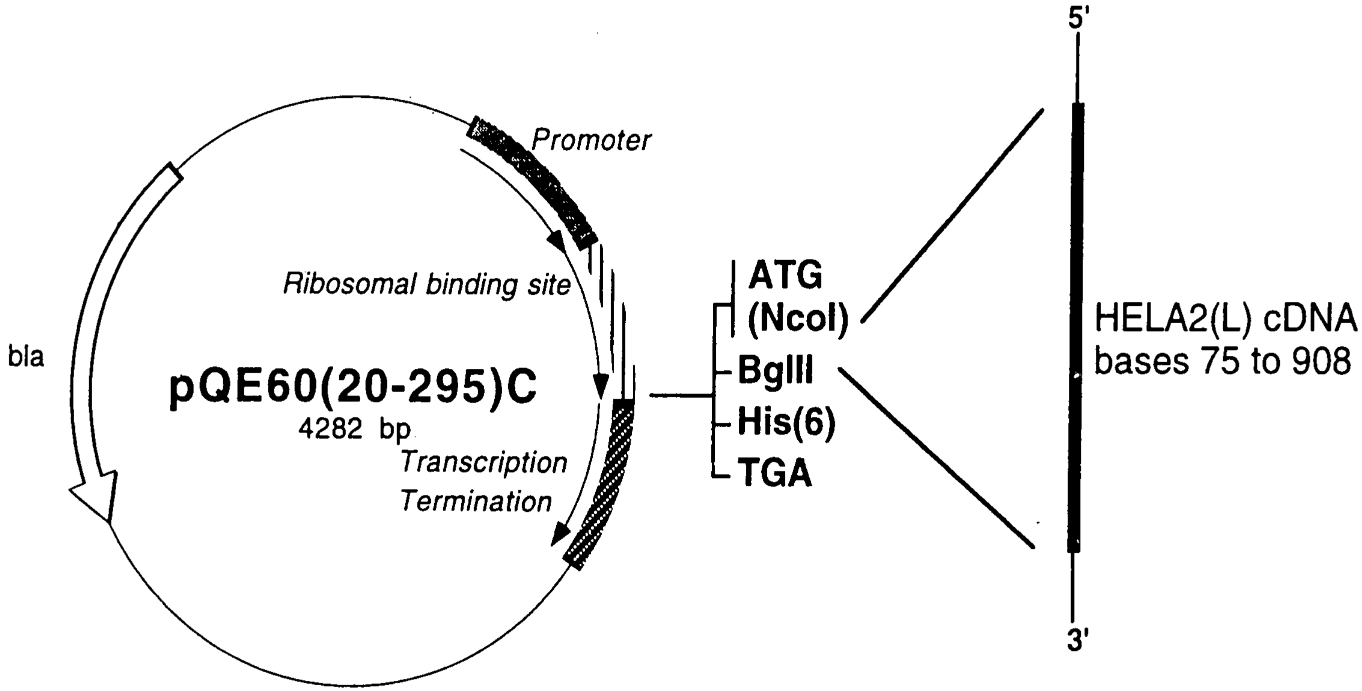

Promoter
Ribosomal binding site
bla
pQE60(20-295)C
4282 bp
Transcription
Termination
ATG
(NcoI)
BglII
His(6)
TGA
5'
3'
HELA2(L) cDNA
bases 75 to 908

FIG 4(ii)

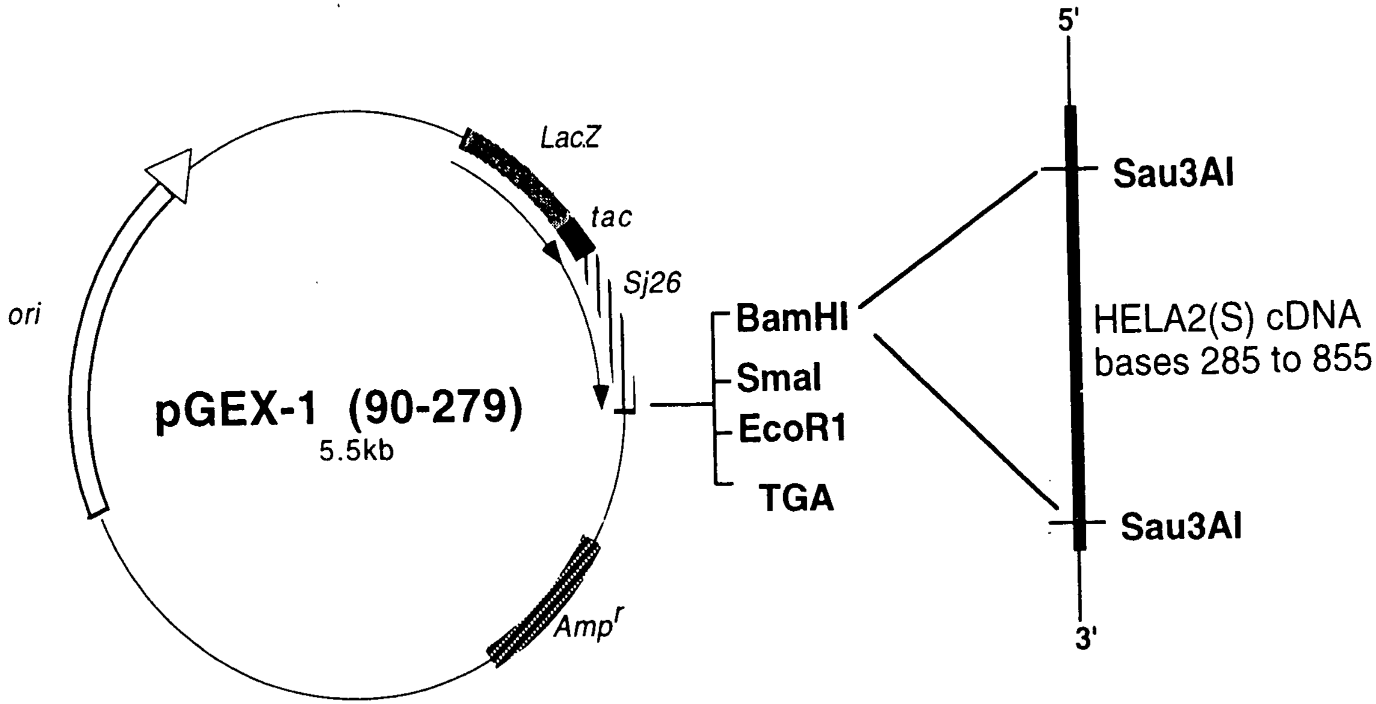
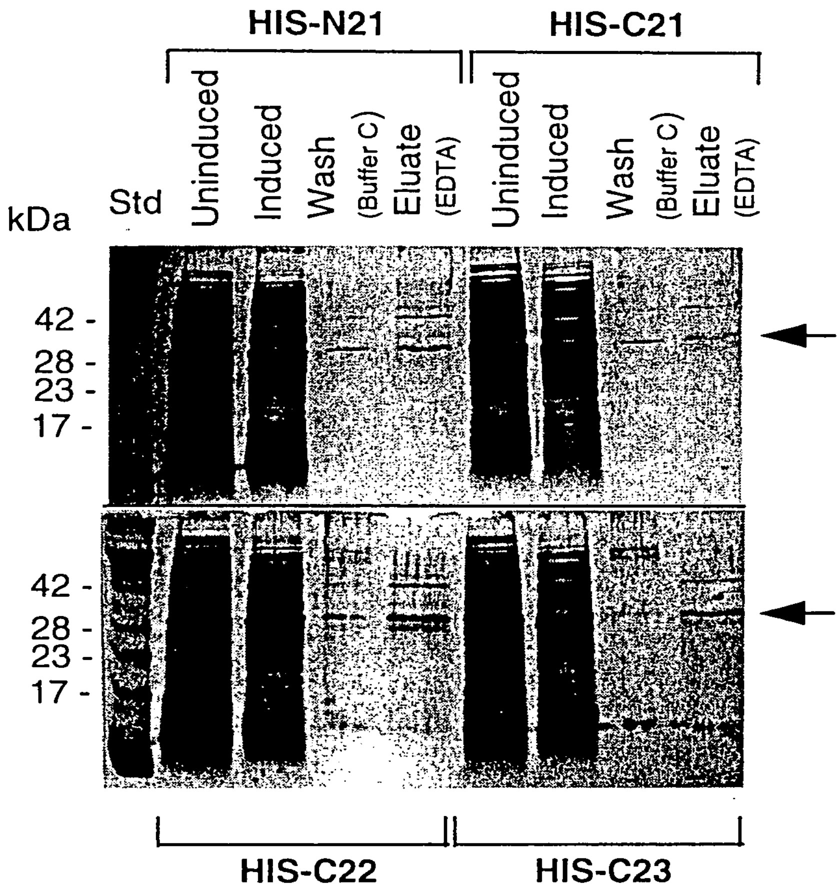

LacZ
tac
Sj26
ori
pGEX-1 (90-279)
5.5kb
Amp^r
BamHI
SmaI
EcoR1
TGA
5'
Sau3AI
HELA2(S) cDNA
bases 285 to 855
Sau3AI
3'

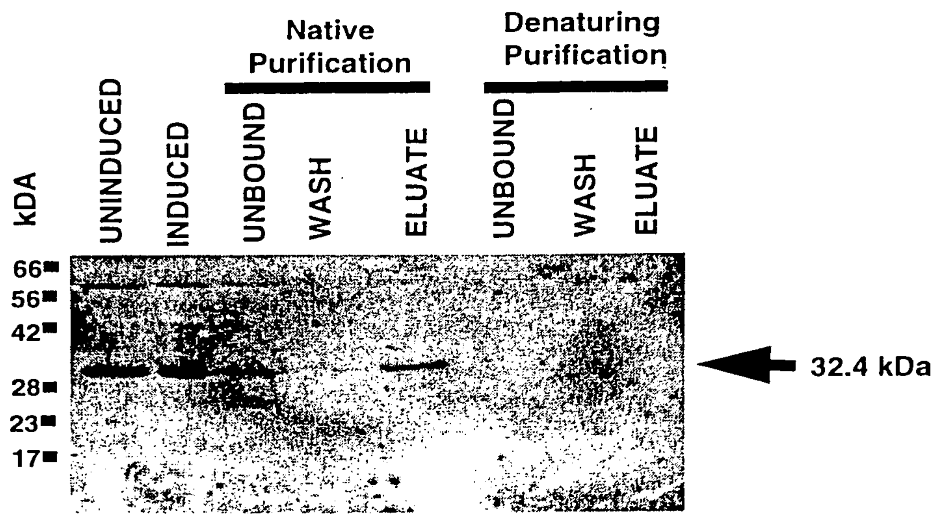
FIG 4(iii)

FIG 5

A. Expression of recombinant Testisin in *E. coli*.



B. Western blot of recombinant Testisin



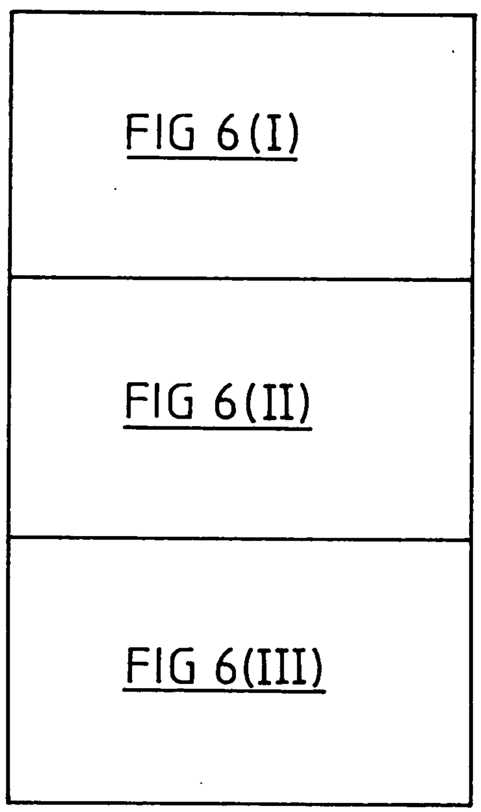


FIG 6

FIGURE 6(I)

```
1                                                   GCCGCGGGAGAGGAGGCC
19   ATGGGCGCGCGGGGCGCTGCTGCTGGCGCTGCTGCTGGCTCGGGCTGGACTCAGGAAG
      M  G  A  R  G  A  L  L  L  A  L  L  L  A  R  A  G  L  R  K    20

79   CCGGAGTCGCAGGAGGCGGCGCCGTTATCAGGACCATGCGGCCGACGGGTCATCACGTCG
      P  E  S  Q  E  A  A  P  L  S  G  P  C  G  R  R  V  I  T  S    40

139  CGCATCGTGGGTGGAGAGGACGCCGAACTCGGGCGTTGGCCGTGGCAGGGGAGCCTGCGC
      R  I  V  G  G  E  D  A  E  L  G  R  W  P  W  Q  G  S  L  R    60

199  CTGTGGGATTCCCACGTATGCGGAGTGAGCCTGCTCAGCCACCGCTGGGCACTCACGGCG
      L  W  D  S  H  V  C  G  V  S  L  L  S  H  R  W  A  L  T  A    80

259  GCGCACTGCTTTGAAACCTATAGTGACCTTAGTGATCCCTCCGGGTGGATGGTCCAGTTT
      A  H  C  F  E  T  Y  S  D  L  S  D  P  S  G  W  M  V  Q  F   100

319  GGCCAGCTGACTTCCATGCCATCCTTCTGGAGCCTGCAGGCCTACTACACCCGTTACTTC
      G  Q  L  T  S  M  P  S  F  W  S  L  Q  A  Y  Y  T  R  Y  F   120

379  GTATCGAATATCTATCTGAGCCCTCGCTACCTGGGGAATTCACCCTATGACATTGCCTTG
      V  S  N  I  Y  L  S  P  R  Y  L  G  N  S  P  Y  D  I  A  L   140
```

FIGURE 6 (II)

```
439  GTGAAGCTGTCTGCACCTGTCACCTACACTAAACACATCCAGCCCATCTGTCTCCAGGCC
      V  K  L  S  A  P  V  T  Y  T  K  H  I  Q  P  I  C  L  Q  A  160

499  TCCACATTTGAGTTTGAGAACCGGACAGACTGCTGGGTGACTGGCTGGGGGTACATCAAA
      S  T  F  E  F  E  N  R  T  D  C  W  V  T  G  W  G  Y  I  K  180

559  GAGGATGAGGCACTGCCATCTCCCCACACCCTCCAGGAAGTTCAGGTCGCCATCATAAAC
      E  D  E  A  L  P  S  P  H  T  L  Q  E  V  Q  V  A  I  I  N  200

619  AACTCTATGTGCAACCACCTCTTCCTCAAGTACAGTTTCCGCAAGGACATCTTTGGAGAC
      N  S  M  C  N  H  L  F  L  K  Y  S  F  R  K  D  I  F  G  D  220

679  ATGGTTTGTGCTGGCAATGCCCAAGGCGGGAAGGATGCCTGCTTCGGTGACTCAGGTGGA
      M  V  C  A  G  N  A  Q  G  G  K  D  A  C  F  G  D  S  G  G  240

739  CCCTTGGCCTGTAACAAGAATGGACTGTGGTATCAGATTGGAGTCGTGAGCTGGGGAGTG
      P  L  A  C  N  K  N  G  L  W  Y  Q  I  G  V  V  S  W  G  V  260

799  GGCTGTGGTCGGCCCAATCGGCCCGGTGTCTACACCAATATCAGCCACCACTTTGAGTGG
      G  C  G  R  P  N  R  P  G  V  Y  T  N  I  S  H  H  F  E  W  280
```

FIGURE 6 (III)

```
859   ATCCAGAAGCTGATGGCCCAGAGTGGCATGTCCCAGCCAGACCCCTCCTGGCCGCTACTC
       I  Q  K  L  M  A  Q  S  G  M  S  Q  P  D  P  S  W  P  L  L  300

919   TTTTTCCCTCTTCTCTGGGCTCTCCCACTCCTGGGGCCGGTCTGAGCCTACCTGAGCCCA 314
       F  F  P  L  L  W  A  L  P  L  L  G  P  V  *

979   TGCAGCCTGGGGCCACTGCCAAGTCAGGCCCTGGTTCTCTTCTGTCTTGTTTGGTAATAA
1039  ACACATTCCAGTTGATGCCTTGCAGGGCATTCTTCAAAAAAAAAAAAAAAAAAAAAAAAA
1099  AAAAAAAAAAAAAAAAAAA
```

Western blot of GST-Testisin using anti-Testisin peptide T175 antibody

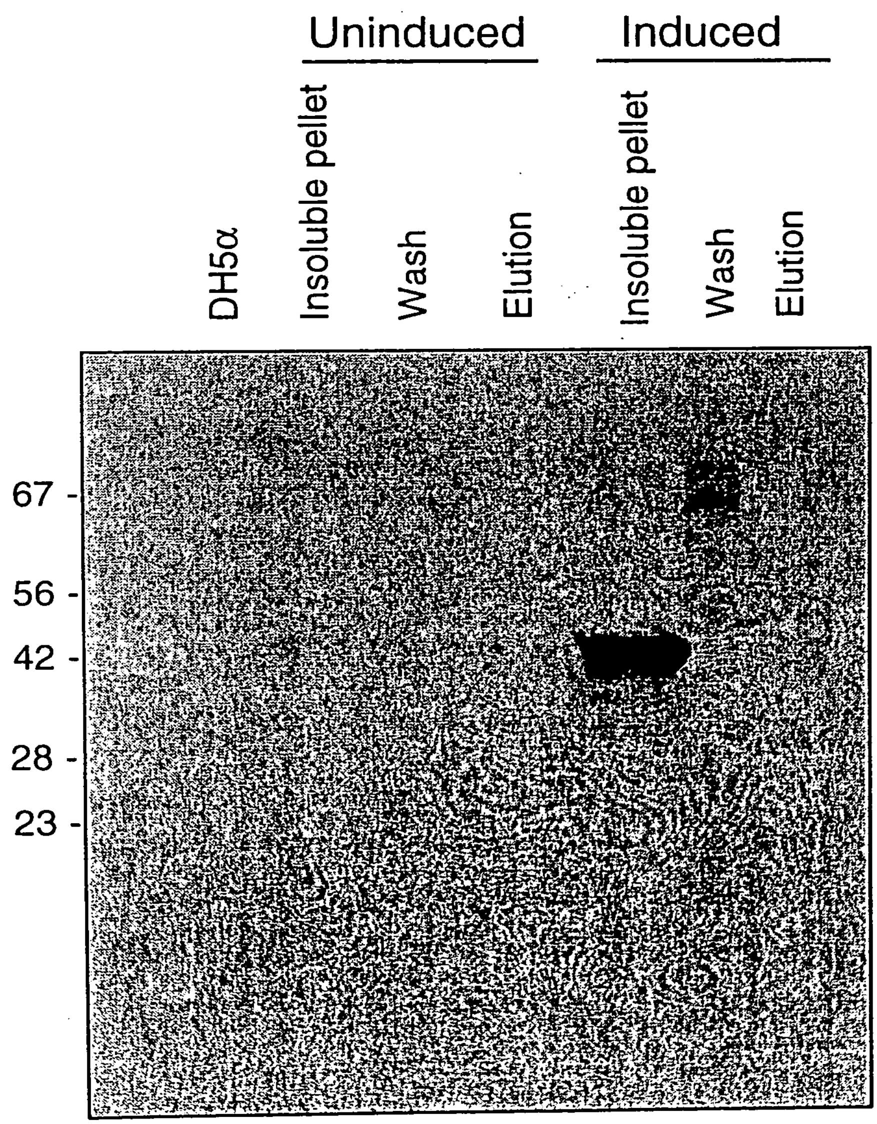
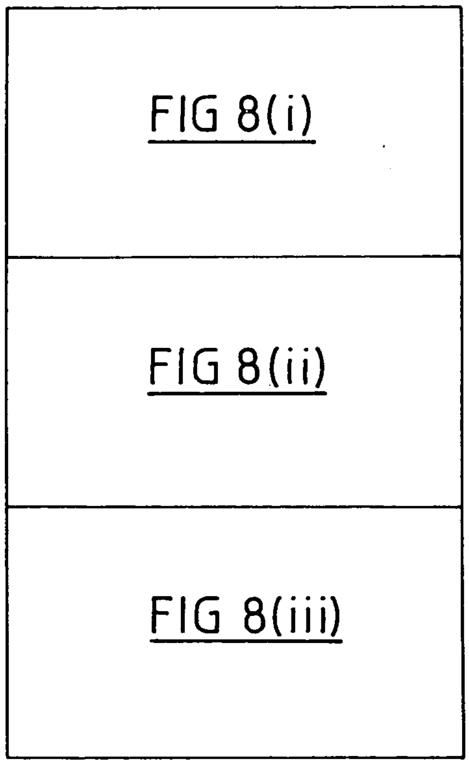
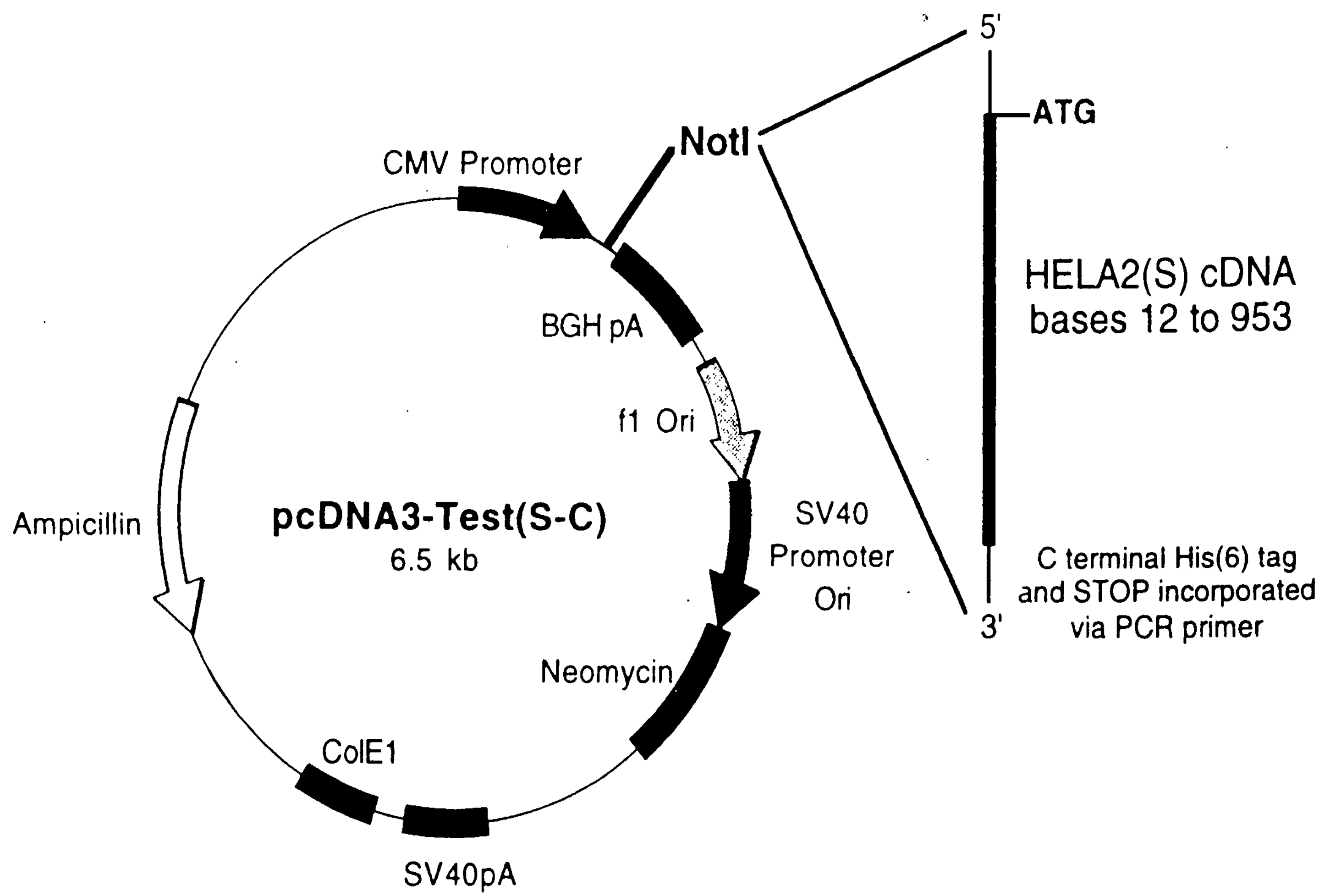


FIG 7

FIG 8





CMV Promoter
NotI
5'
ATG
HELA2(S) cDNA
bases 12 to 953
C terminal His(6) tag
and STOP incorporated
via PCR primer
3'
BGH pA
f1 Ori
SV40
Promoter
Ori
Neomycin
SV40pA
ColE1
Ampicillin
pcDNA3-Test(S-C)
6.5 kb

FIG 8(i)

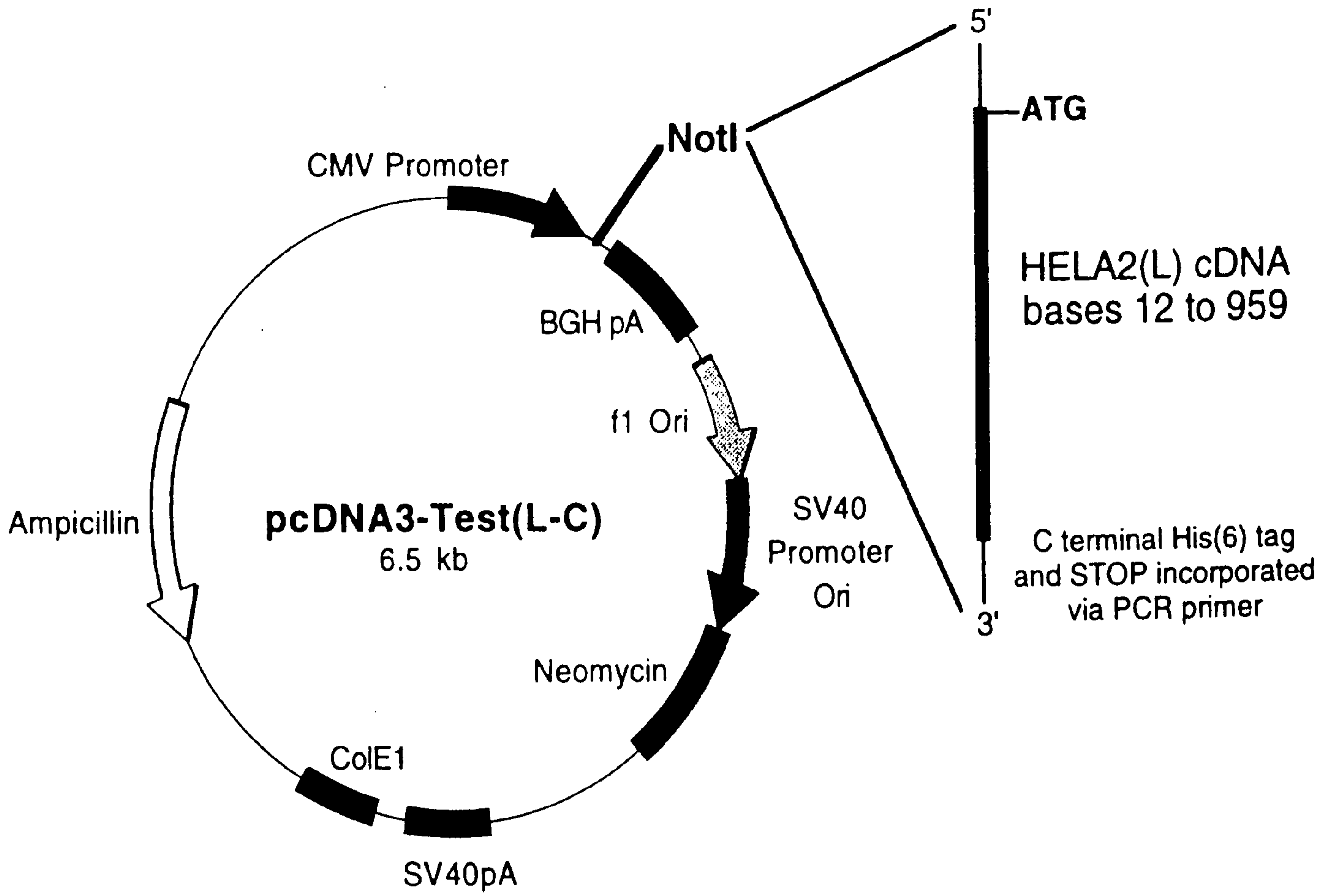

CMV Promoter
NotI
5'
ATG
HELA2(L) cDNA
bases 12 to 959
C terminal His(6) tag
and STOP incorporated
via PCR primer
3'
BGH pA
f1 Ori
SV40
Promoter
Ori
Ampicillin
pcDNA3-Test(L-C)
6.5 kb
Neomycin
ColE1
SV40pA

FIG 8(ii)

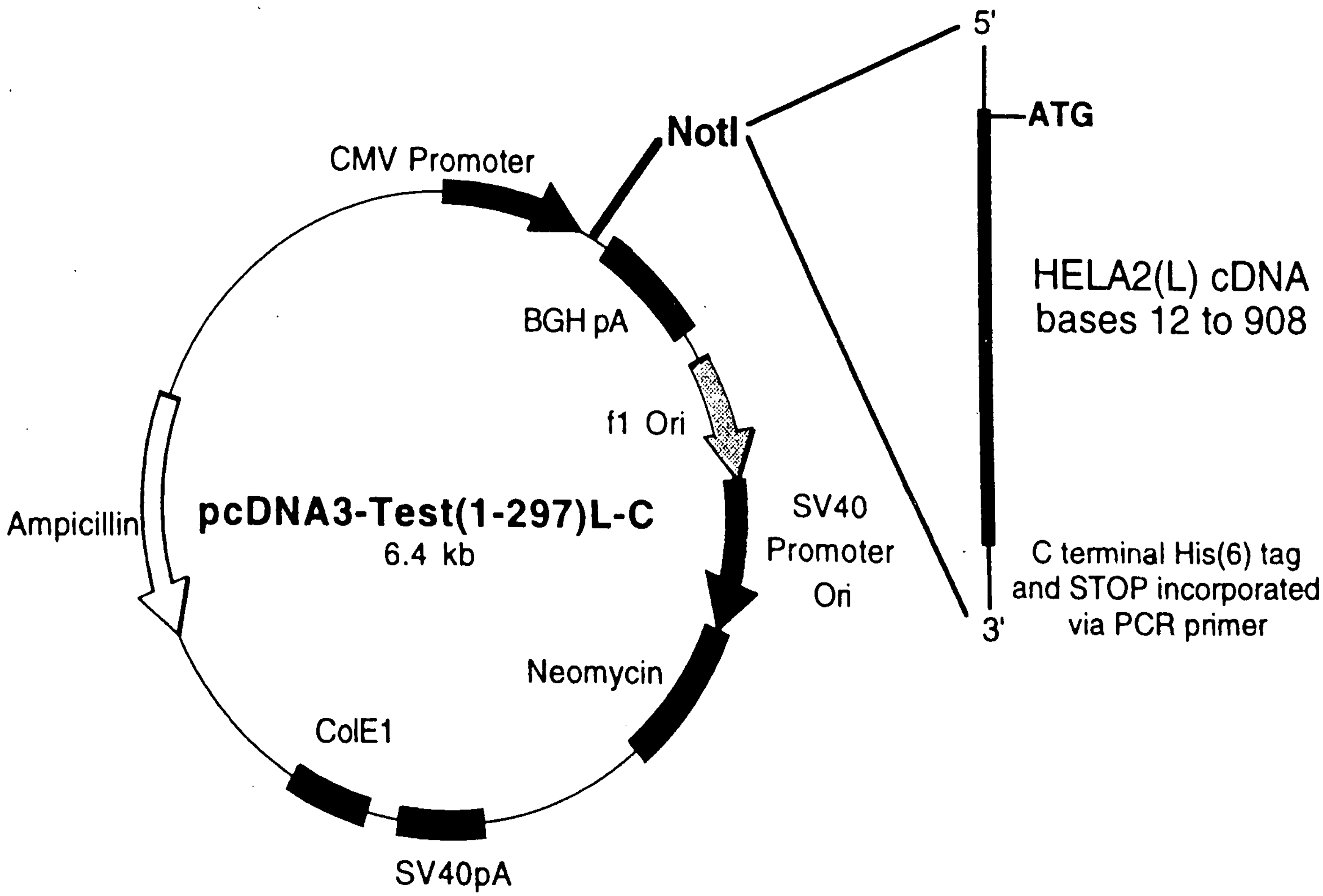

CMV Promoter
NotI
5'
ATG
HELA2(L) cDNA
bases 12 to 908
C terminal His(6) tag
and STOP incorporated
via PCR primer
3'
BGH pA
f1 Ori
SV40
Promoter
Ori
pcDNA3-Test(1-297)L-C
6.4 kb
Ampicillin
Neomycin
ColE1
SV40pA

FIG 8(iii)

FIG 9

FIG 9(i)	FIG 9(ii)
FIG 9(iii)	FIG 9(iv)

FIG 9(i)

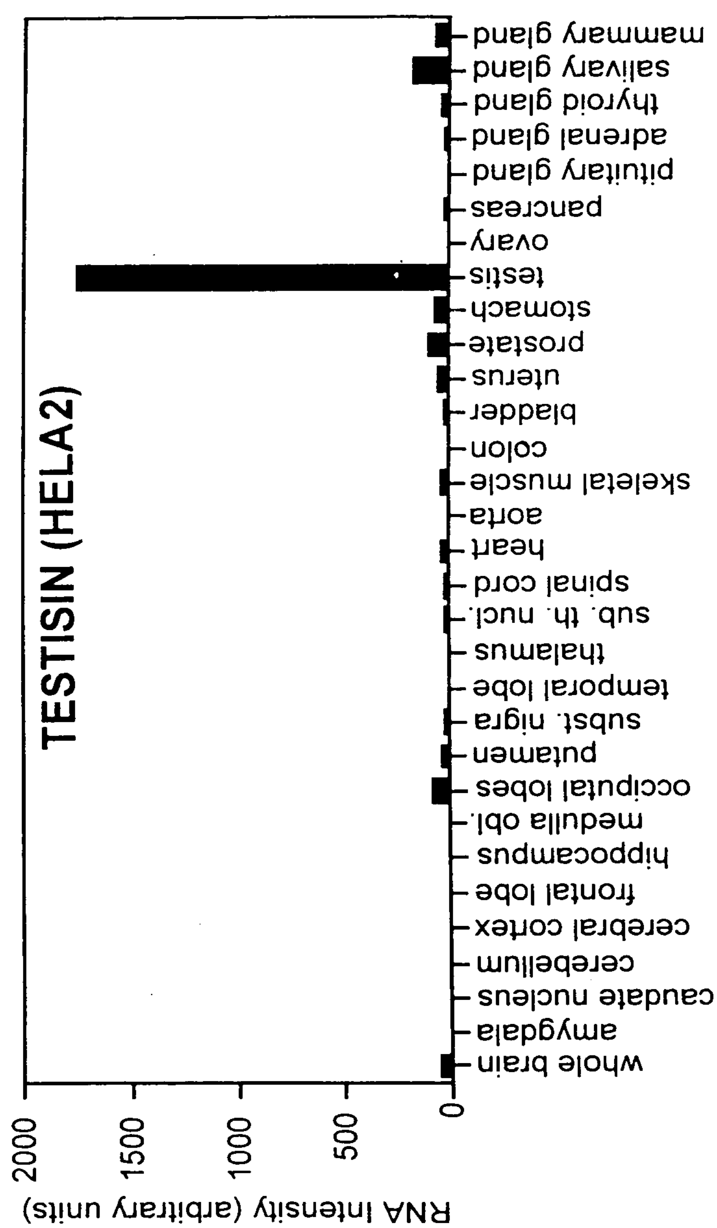
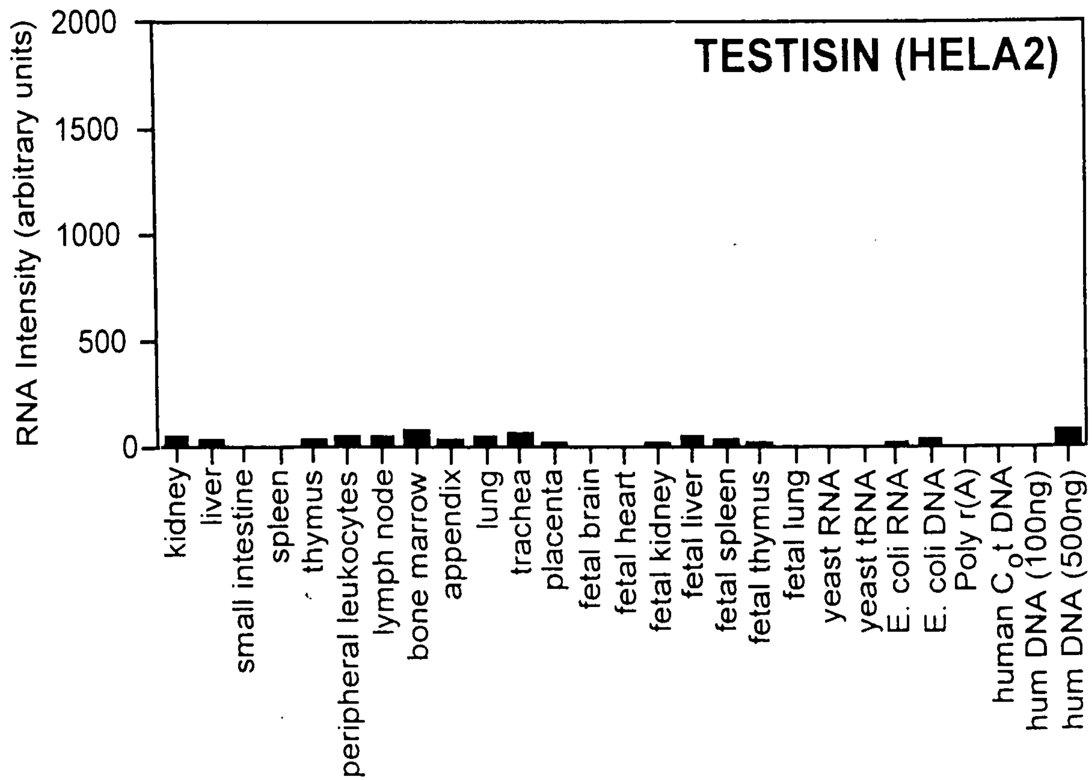

TESTISIN (HELA2)
RNA Intensity (arbitrary units)
0
500
1000
1500
2000
whole brain
amygdala
caudate nucleus
cerebellum
cerebral cortex
frontal lobe
hippocampus
medulla obl.
occipital lobes
putamen
subst. nigra
temporal lobe
thalamus
sub. th. nucl.
spinal cord
heart
aorta
skeletal muscle
colon
bladder
uterus
prostate
stomach
testis
ovary
pancreas
pituitary gland
adrenal gland
thyroid gland
salivary gland
mammary gland

FIG 9(ii)


TESTISIN (HELA2)
RNA Intensity (arbitrary units)
2000
1500
1000
500
0
kidney
liver
small intestine
spleen
thymus
peripheral leukocytes
lymph node
bone marrow
appendix
lung
trachea
placenta
fetal brain
fetal heart
fetal kidney
fetal liver
fetal spleen
fetal thymus
fetal lung
yeast RNA
yeast tRNA
E. coli RNA
E. coli DNA
Poly r(A)
human C_ot DNA
hum DNA (100ng)
hum DNA (500ng)

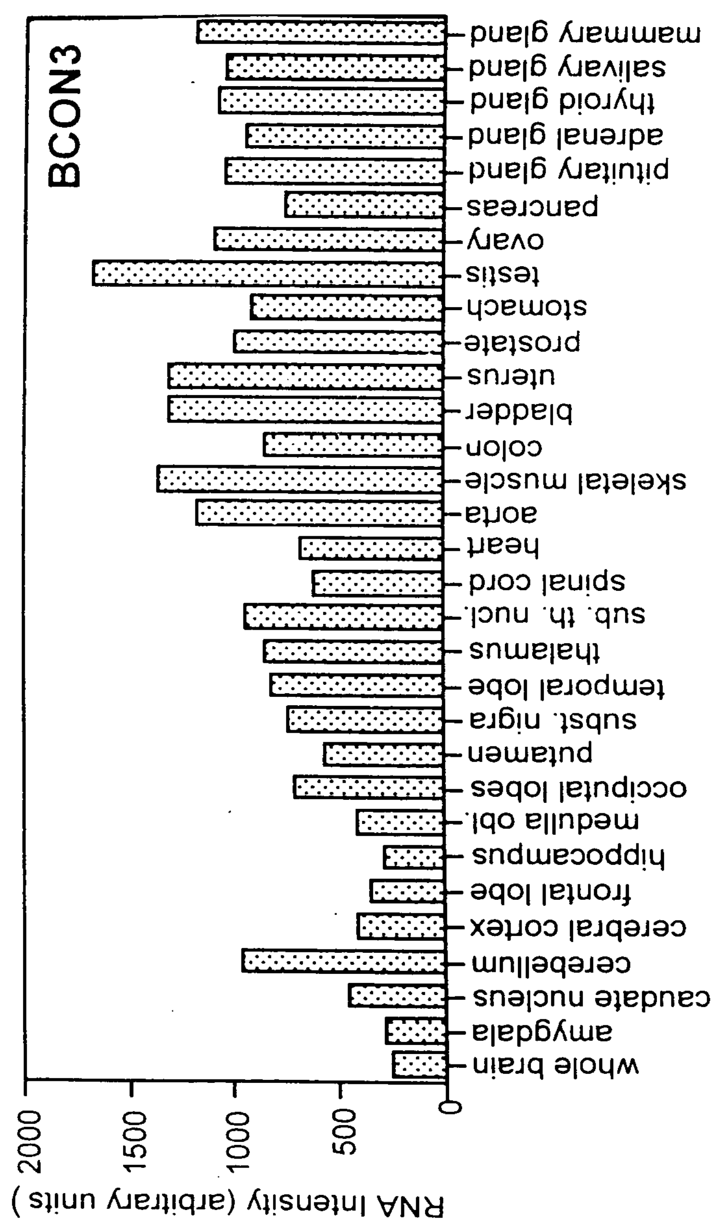

BCON3
RNA Intensity (arbitrary units)
0
500
1000
1500
2000
whole brain
amygdala
caudate nucleus
cerebellum
cerebral cortex
frontal lobe
hippocampus
medulla obl.
occipital lobes
putamen
subst. nigra
temporal lobe
thalamus
sub. th. nucl.
spinal cord
heart
aorta
skeletal muscle
colon
bladder
uterus
prostate
stomach
testis
ovary
pancreas
pituitary gland
adrenal gland
thyroid gland
salivary gland
mammary gland

FIG 9(iii)

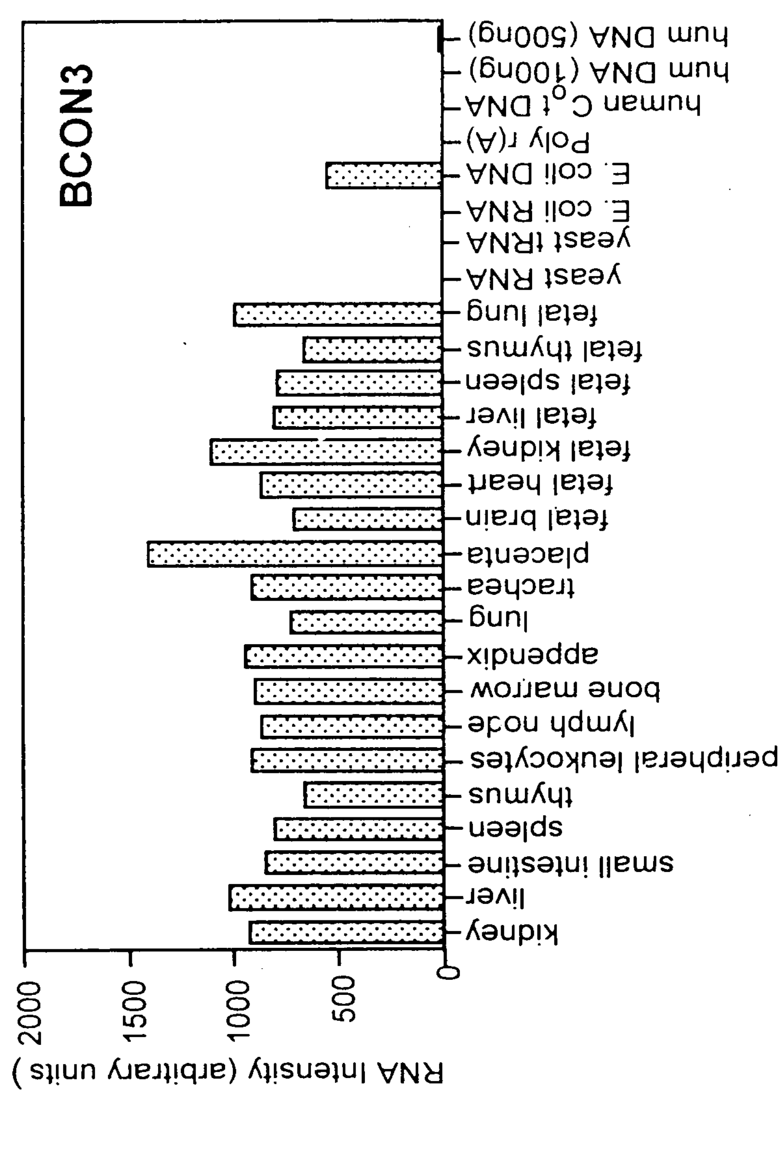

BCON3
RNA Intensity (arbitrary units)
0
500
1000
1500
2000
kidney
liver
small intestine
spleen
thymus
peripheral leukocytes
lymph node
bone marrow
appendix
lung
trachea
placenta
fetal brain
fetal heart
fetal kidney
fetal liver
fetal spleen
fetal thymus
fetal lung
yeast RNA
yeast tRNA
E. coli RNA
E. coli DNA
Poly r(A)
human C_0t DNA
hum DNA (100ng)
hum DNA (500ng)

FIG 9(iv)

FIG 10

A.

HELA2 (Testisin)

B.

BCON3

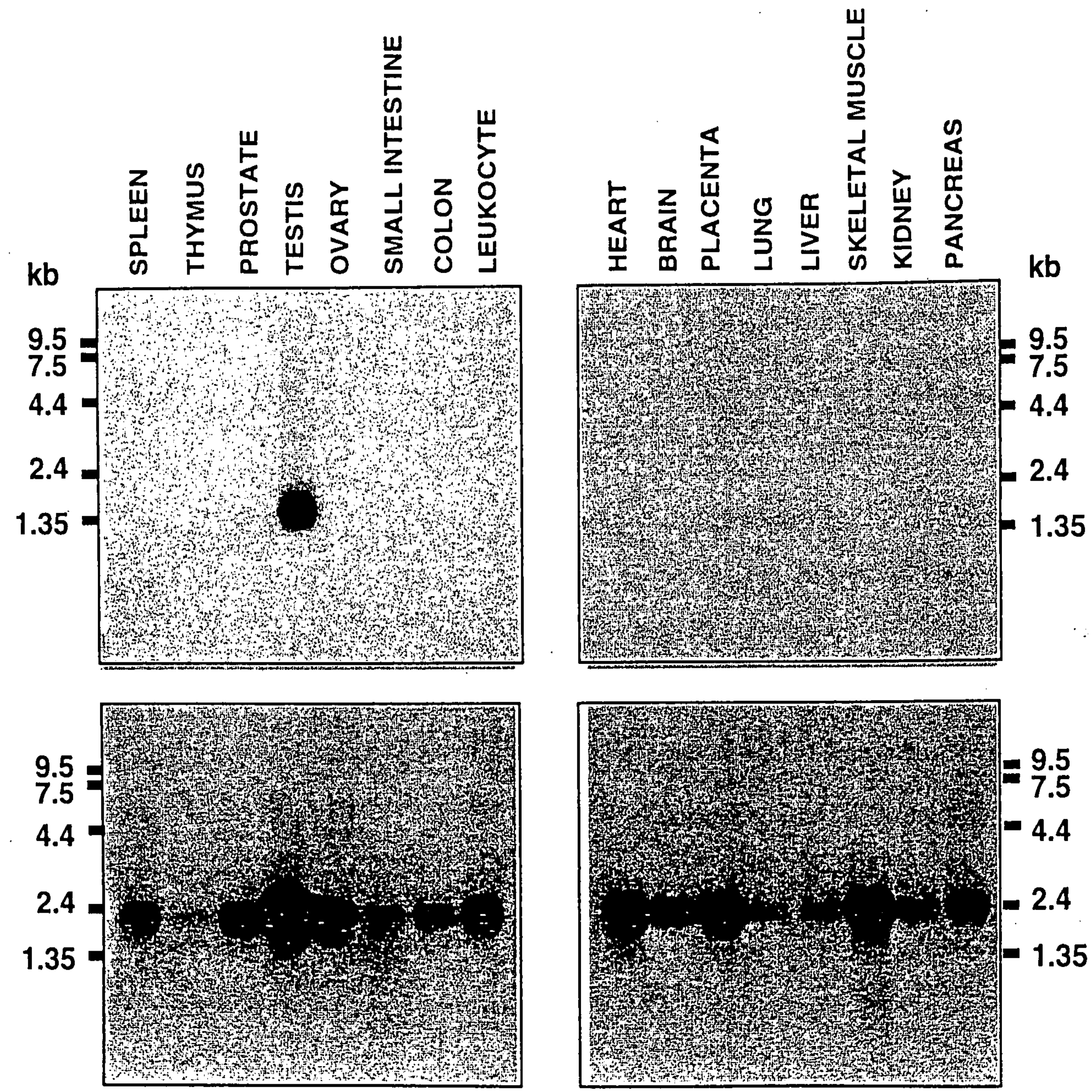
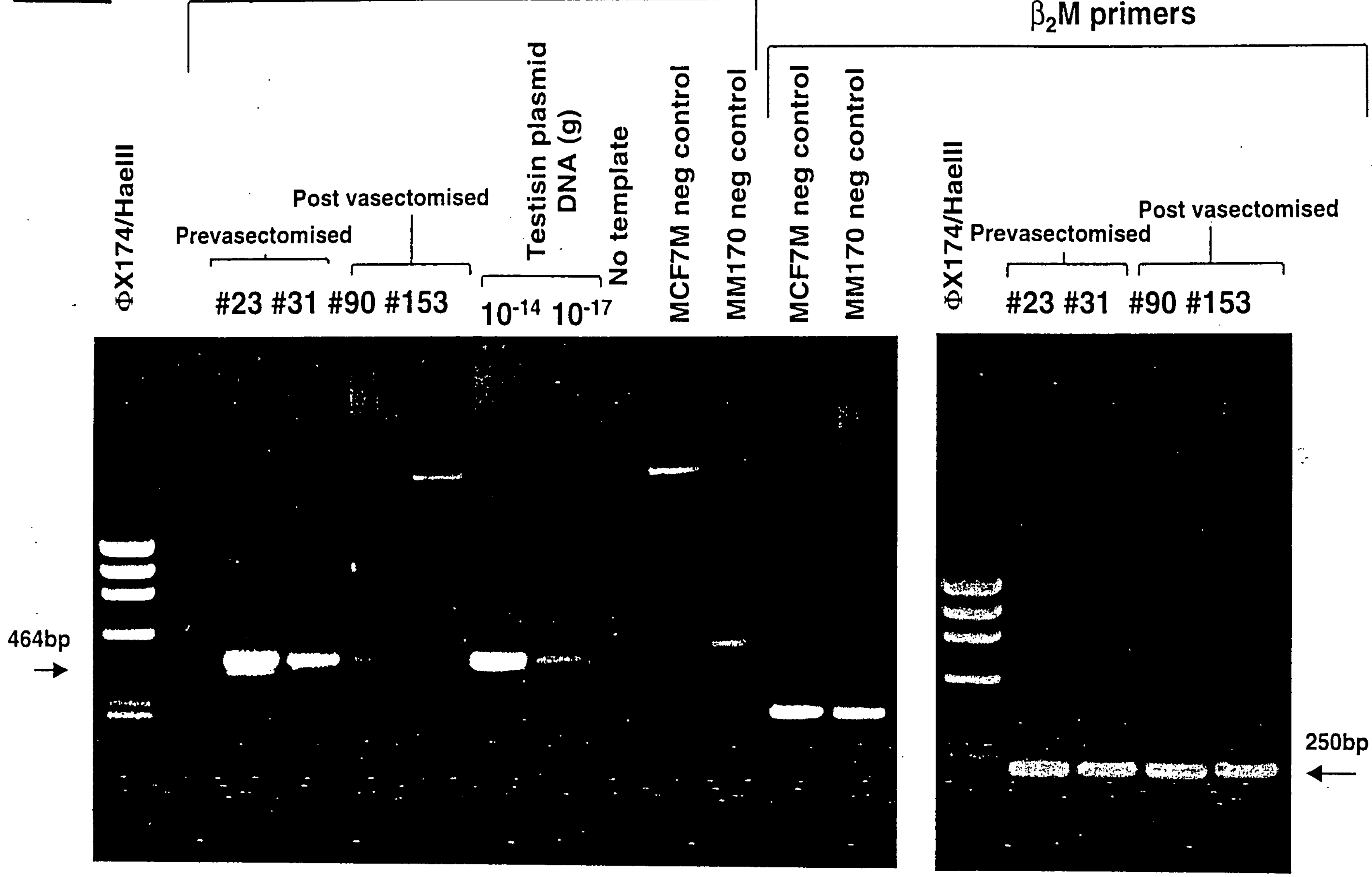


FIG 11


Testisin primers P8 and P9
β2M primers
ΦX174/HaeIII
Prevasectomised
Post vasectomised
#23 #31 #90 #153
Testisin plasmid DNA (g)
10-14 10-17
No template
MCF7M neg control
MM170 neg control
MCF7M neg control
MM170 neg control
ΦX174/HaeIII
Prevasectomised
Post vasectomised
#23 #31 #90 #153
464bp
250bp

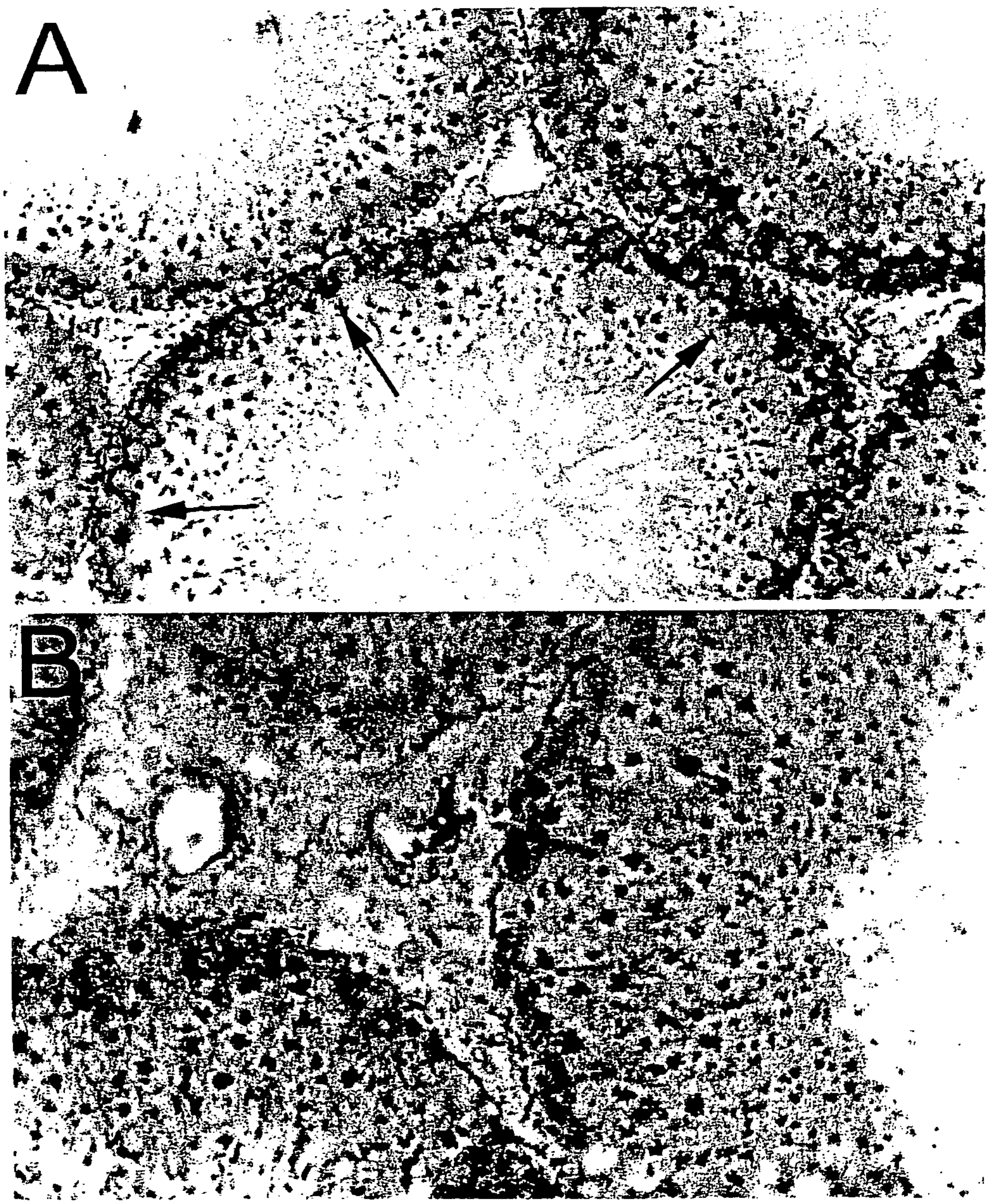


FIG 12

Testisin (HELA2) is located on human chromosome 16p13.3

A

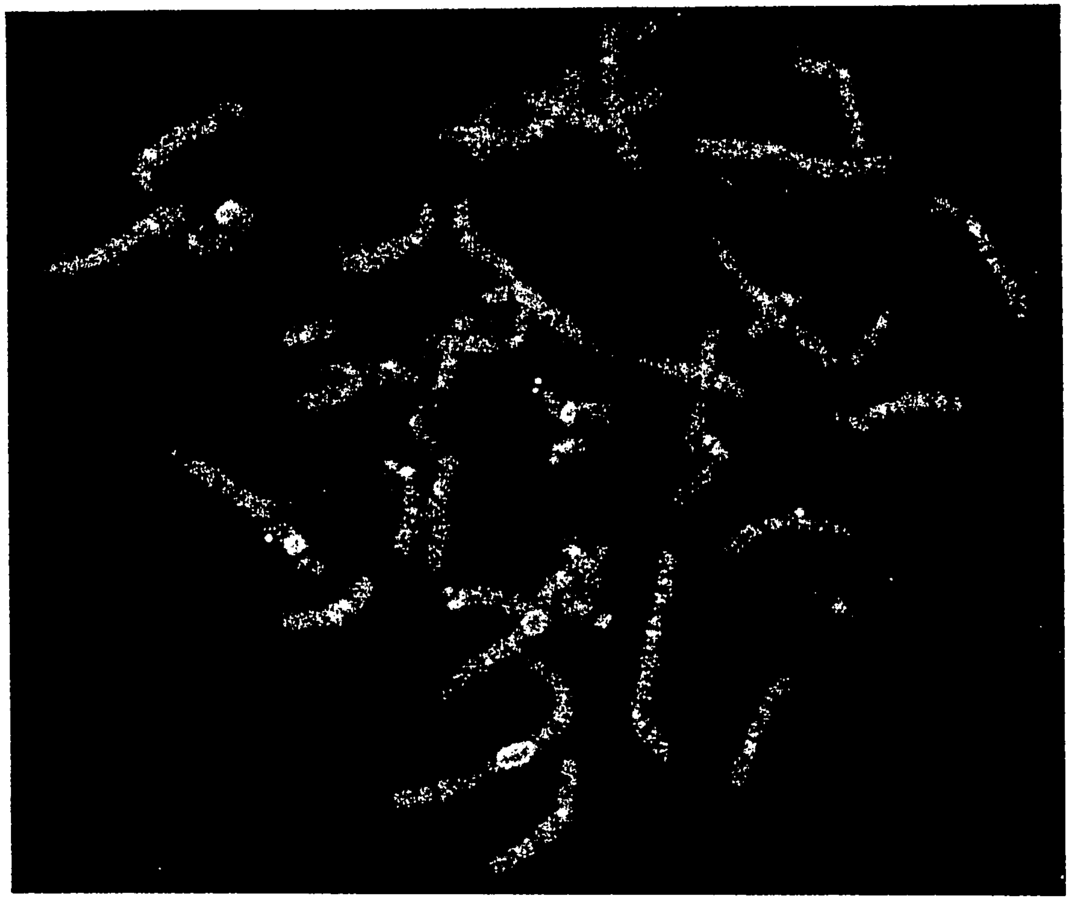


FIG 13A

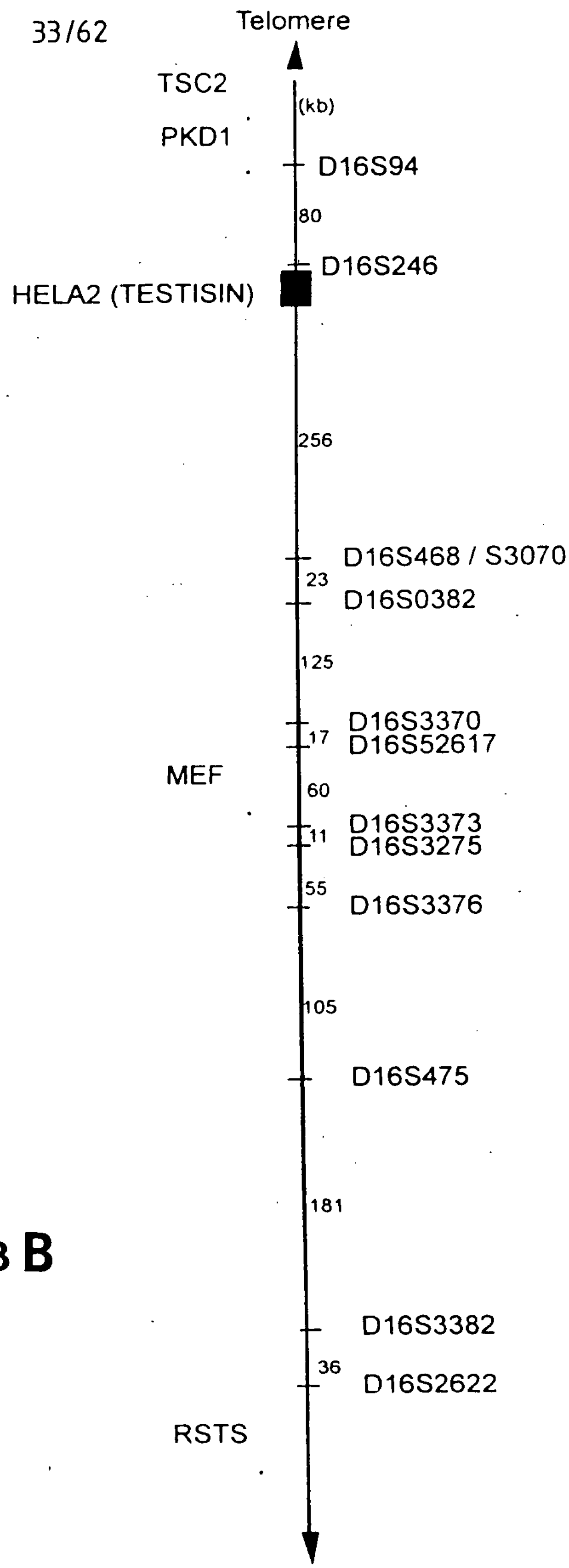
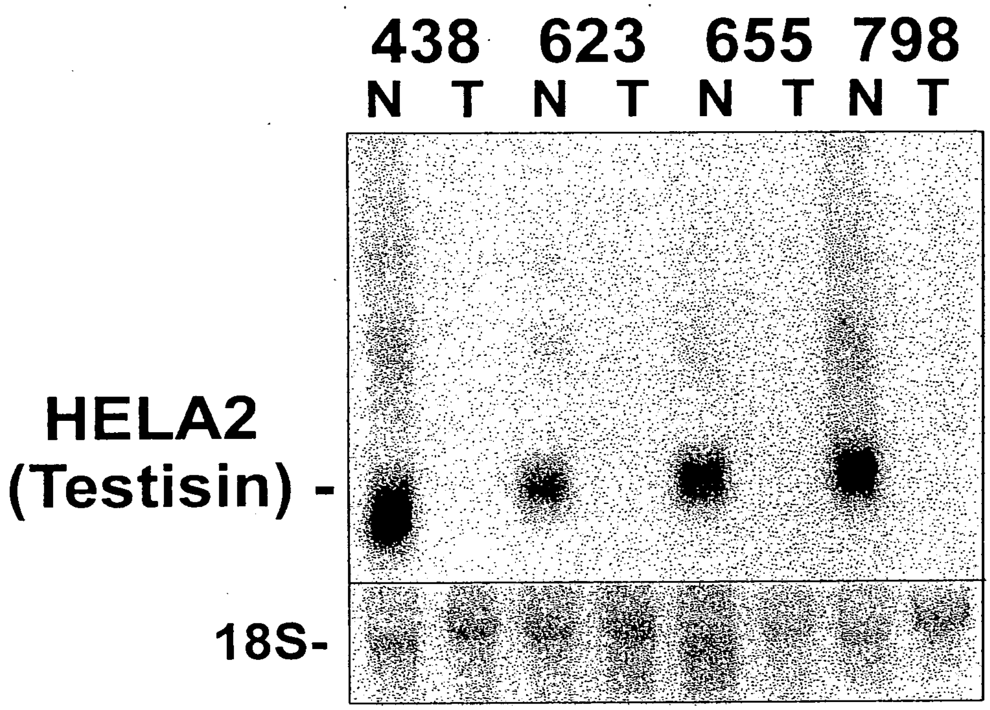

Telomere
TSC2
(kb)
PKD1
D16S94
80
D16S246
HELA2 (TESTISIN)
256
D16S468 / S3070
23
D16S0382
125
D16S3370
17
D16S52617
MEF
60
D16S3373
11
D16S3275
55
D16S3376
105
D16S475
181
D16S3382
36
D16S2622
RSTS

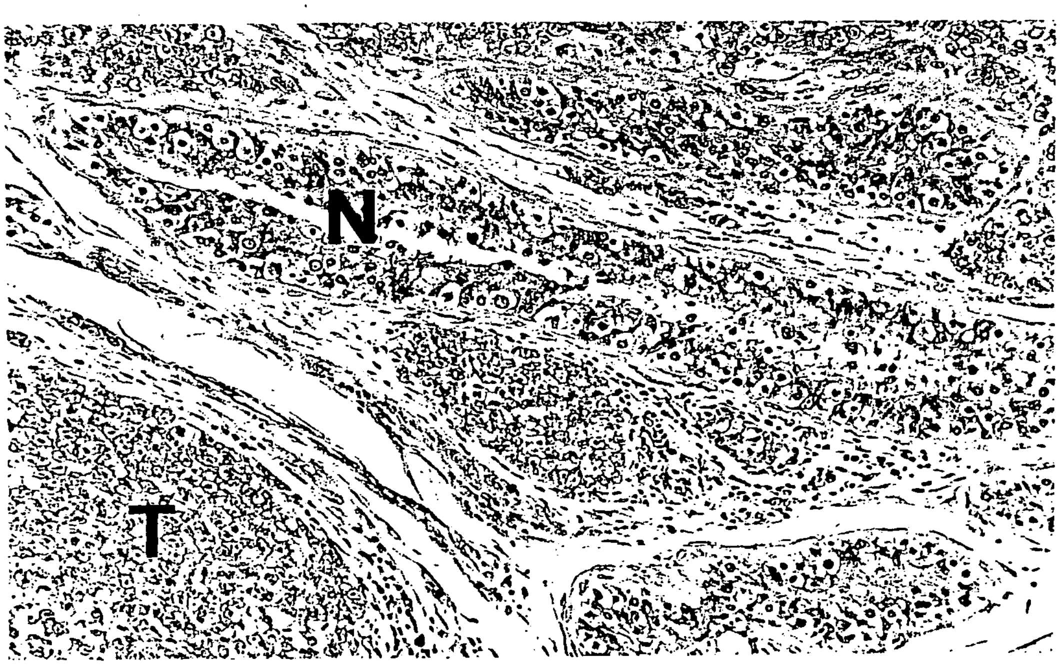
FIGURE 13 B

FIG 14

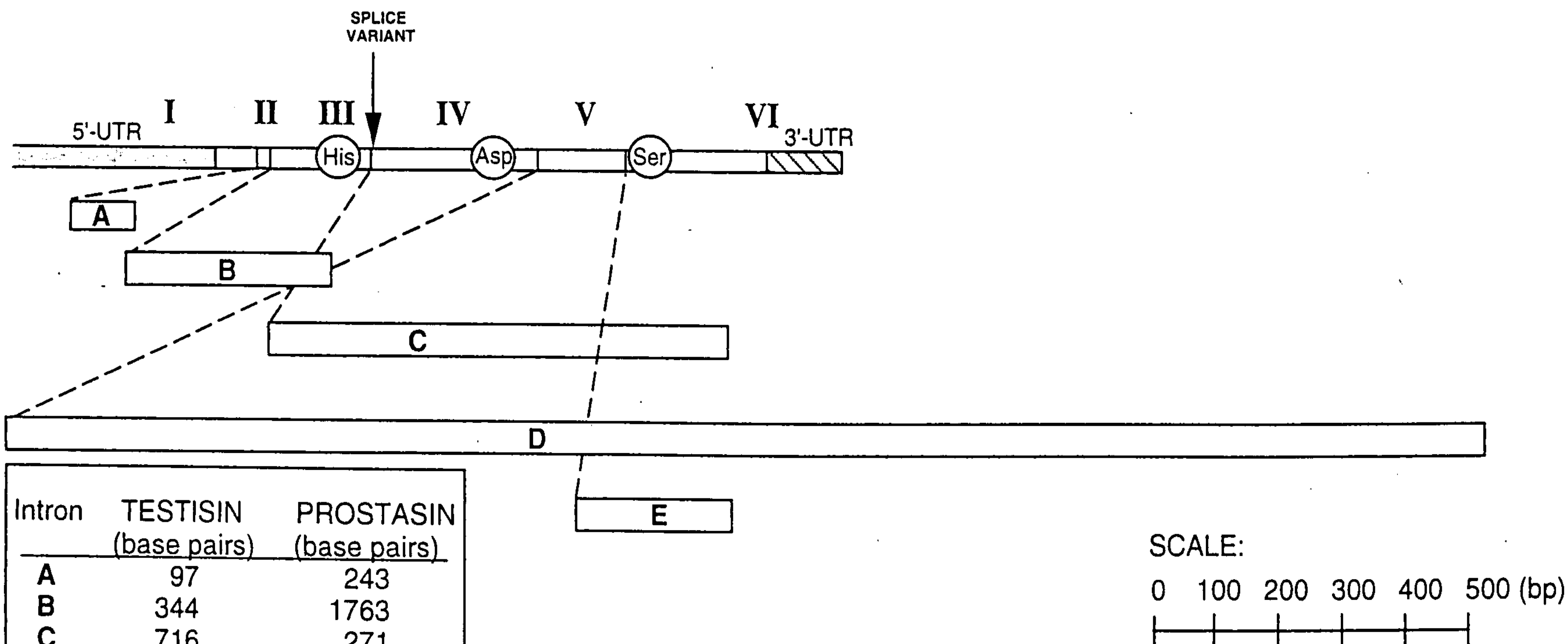
A. Northern Blot



B. Immunohistochemistry



TESTISIN INTRON/EXON BOUNDARIES AND SIZES

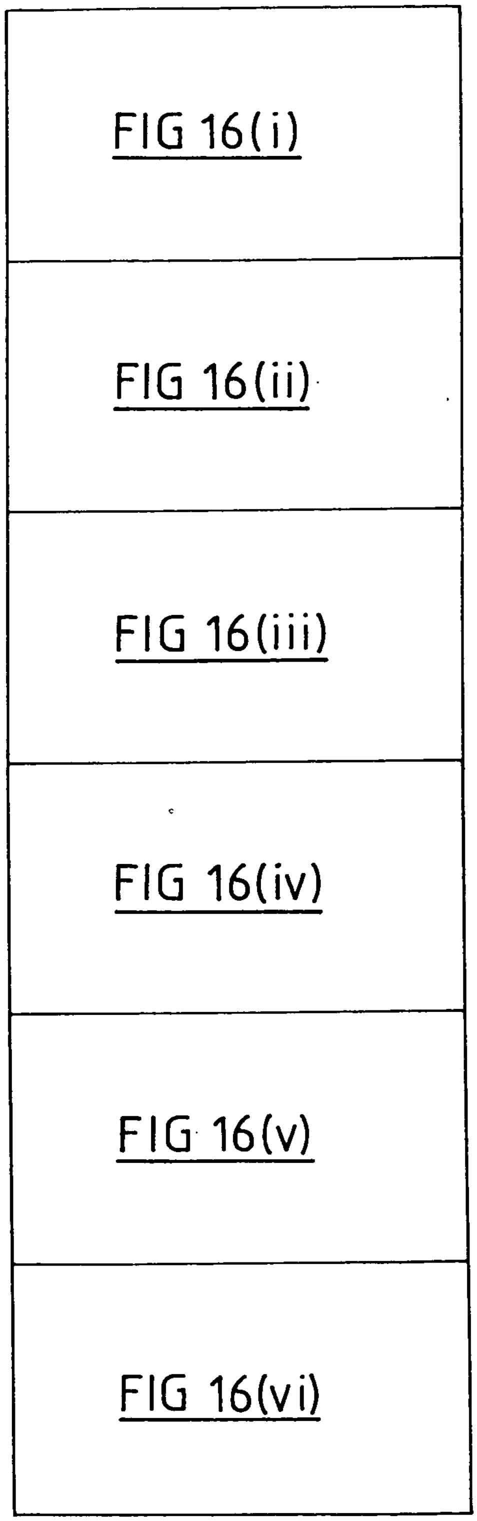


Intron	TESTISIN (base pairs)	PROSTASIN (base pairs)
A	97	243
B	344	1763
C	716	271
D	~2200	85
E	256	92

Exon	TESTISIN (base pairs)	PROSTASIN (base pairs)
I	>76	417
II	18	18
III	163	163
IV	284	272
V	168	167
VI	348	899

FIGURE 15

FIG 16



```
agtgagtctc ctgcctcagc ctcccaagta gctgggactt caggtgtgtg      50
ccaccatcct cagctaattt tttttttttt ttttttttg agaaggagtc       100
ttgctctgtc gcccaggctg gagtgcagtg gcgcgatctt ccaggcccca      150
ccgggccctc aggaaggcct tgcctacctg ctttaagggg actcctggct      200
cagggccagg cccctggtgc tggaggaggt ggtgggtgga gggcaggggg      250
caccaagcgg gcagccagga cccccgggct gcagacaaga aaaggactgt      300
                      /+1...EXON 1...
ggggtccacc gggtctgggc cACATCAAGG AATGTGGTTG AAGACCCGCC      350
CTTAGGAGCT GAAAGCCAGG GCGCTACCAG GCCTGAGAGG CCCCAAACAG      400
CCCTTGGGCC TGGTTTGGGA GGATTAAGCT GGAGCTCCCA ACCCGCCCTG      450
CCCCCAGGGG GCGACCCCGG GCCCGGCGCG AGAGGAGGCA GAGGGGGCGT      500
CAGGCCGCGG GAGAGGAGGC CATGGGCGCG CGCGGGGCGC TGCTGCTGGC      550
                                         /INTRON A...
GCTGCTGCTG GCTCGGGCTG GACTCAGGAA GCCGGgtgag ctcggggcgc      600
tgctggcggg atggggaggc gggggagcgg tggggaggac gggaggtgga      650
```

FIG 16(i)

```
                                       /EXON 2...
ggccgcgggg agtcacttct tgtctcccgc agAGTCGCAG GAGGCGGCGC      700
          /INTRON B...
CGTTATCAGg tagggcgccc aggacgcgcg attcctgcca gggccgttgg      750
gccgaggtgg acgggggcg gtgaggggt agagggggc ctttactgct      800
ctctcgcccc cgcccccggg atcgagaact ctgttggcgt ggaaagtaac      850
taacggacgc tggaggggga tgggcgggcc ctgcagagca cgtgggagga      900
tctccagtgt cacctacttc ctgctgcaca cacgcgaggg gaccctgggt      950
gggcaaaaac gtgctttccc ggacggggtt gaaggggaga aagggagagg     1000
tcgggcttgg ggggctgcct cccgcggctc agcagttcct ctgaccatcc     1050
    /EXON 3...
gagGACCATG CGGCCGACGG GTCATCACGT CGCGCATCGT GGGTGGAGAG     1100
GACGCCGAAC TCGGGCGTTG GCCGTGGCAG GGGAGCCTGC GCCTGTGGGA     1150
TTCCCACGTA TGCGGAGTGA GCCTGCTCAG CCACCGCTGG GCACTCACGG     1200
```

FIG 16(ii)

/INTRON C...

```
CGGCGCACTG CTTTGAAACg tgagtggggg tgcgaacgga ggggtgcggg 1250
gacgggcagg aacagggctg gagggagtgc caccgaactt tacctctggt 1300
ctgatgccag acttgggcgt gaaagttgtg cgtggatgcg gcctggtgtt 1350
ctcctgagcc ccaggctgtg ctgcagccgg ttacacccac tccagttccc 1400
tttgggtctc ctggagggaa ccctgttcag gttattccag aatgttcttc 1450
cagaacattt ccacacactt ttgggtattc tctccctttt tctttcaacc 1500
caaagttcac cactgaccat cccaccctca tccccccctcc tggtggacgg 1550
tgcggtacag tgtggggcac tgagccaagg ccagcacccc cgggccgctg 1600
tgtggactcc atcctgccaa tcccacattg gcgtggtgca tctccccatt 1650
cctccttggg ctgcatgggg gtgcccctgg aggccttggc tcaatgcaag 1700
gctccttggg acagctctgg gaggtgacaa gaccccaccc ttctgctgca 1750
ggagcaggtc ctaggacttt ggttgtggtc tgtctgggct ccttcatttc 1800
tgcaggggac cctgggtgtt agcaagtagc agcaacacca cagtttcccc 1850
tcctgcactg gaccccagtt gtgctcaggt agccagccct ccatccaggg 1900
```

FIG 16(iii)

```
                                            /EXON 4...
ccccctgactg ctctcttctc ttctgccagc tatagTGACC TTAGTGATCC   1950
CTCCGGGTGG ATGGTCCAGT TTGGCCAGCT GACTTCCATG CCATCCTTCT    2000
GGAGCCTGCA GGCCTACTAC ACCCGTTACT TCGTATCGAA TATCTATCTG    2050
AGCCCTCGCT ACCTGGGGAA TTCACCCTAT GACATTGCCT TGGTGAAGCT    2100
GTCTGCACCT GTCACCTACA CTAAACACAT CCAGCCCATC TGTCTCCAGG    2150
CCTCCACATT TGAGTTTGAG AACCGGACAG ACTGCTGGGT GACTGGCTGG    2200
                            /INTRON  D...
GGGTACATCA AAGAGGATGA GGgtgaggct ggggacaggc gggtcaggga    2250
ggaactgtct ttgttcacct gttcccctgc ataggcacaa tagccccctg    2300
cttggtctgg gggtgcaggc tatgcccctc ttgcttgcag tctctcctca    2350
cctgccaggg cagggaccaa acacccagtt ctctcccttc caggggctgt    2400
gggggccaga aggagagtgt gagagggagg ccagtttggc gcaagcctgt    2450
gggtggtgcg gtggtggagg ggttctggag ggcttggcga cataaacctc    2500
atacttggat ttattcctgc atctttccac ctcccccagt gctcaccaat    2550
```

FIG 16(iv)

```
gccccaggca tca..............approx 1000 bp...........        3563
ccaggttgcc ccttccccca aggtctggct ttggatgctt atgtgaacac     ≈3613
cgttttaagt tgccttggcc ccttcctcgg ttcctttttg gctgaggaat     ≈3663
ctctccatgg ctgcaggcag ggccattgtt gccattctac agatagggaa     ≈3713
agtgcggctg ggggagctct gacagctgtc cctccccggg gccttctgtg     ≈3763
atgctgctga gggcctctgt tgtgctgggg tctgggttgg agctgggggt     ≈3813
aatggagatg aacctgccag gcacagtggg tgccccaggg cccccacccc     ≈3863
cgcagcctat gccatccctc catagagggg cctcaggttg ctgtctctct     ≈3913
                          /EXON 5...
ccttcccact atcgtccgca cagCACTGCC ATCTCCCCAC ACCCTCCAGG     ≈3963
AAGTTCAGGT CGCCATCATA AACAACTCTA TGTGCAACCA CCTCTTCCTC     ≈4013
AAGTACAGTT TCCGCAAGGA CATCTTTGGA GACATGGTTT GTGCTGGCAA     ≈4063
                                /INTRON  E...
TGCCCAAGGC GGGAAGGATG CCTGCTTCgt gagtgtcctt gccaccactc     ≈4113
ccagcccagg aaagcatcct gtgtccctgt gccttatttg accctcatgc     ≈4163
caaccccggg aggtggagac tgttgcccca ctctgcagat gcagaaacgg     ≈4213
```

FIG 16(v)

```
aggcttggct gctgccaggg ggaggaggag gatgtgcacc cagtctaccc   ≈4263
agccccatag cccttcccac tctcagcccc tcccctgccc cactcactct   ≈4313
                                          /EXON 6...
gccccaggct gacctcagcc ccgctgctcc ccagGGTGAC TCAGGTGGAC   ≈4363
CCTTGGCCTG TAACAAGAAT GGACTGTGGT ATCAGATTGG AGTCGTGAGC   ≈4413
TGGGGAGTGG GCTGTGGTCG GCCCAATCGG CCCGGTGTCT ACACCAATAT   ≈4463
CAGCCACCAC TTTGAGTGGA TCCAGAAGCT GATGGCCCAG AGTGGCATGT   ≈4513
CCCAGCCAGA CCCCTCCTGG CCGCTACTCT TTTTCCCTCT TCTCTGGGCT   ≈4563
CTCCCACTCC TGGGGCCGGT CTGAGCCTAC CTGAGCCCAT GCAGCCTGGG   ≈4613
GCCACTGCCA AGTCAGGCCC TGGTTCTCTT CTGTCTTGTT TGGTAATAAA   ≈4663
CACATTCCAG TTGATGCCTT GCAGGGCATT CTTCAaaagc agtggcttca   ≈4713
tggacagctc attctctctt gtgcagacag cctgtctgtg cccctggctc   ≈4763
acacccacat ctgttctgca ccatagaacc atctggttat ttcgatcaga   ≈4813
aagagaattg tgtgttgccc aggctggtct tgaacgccta gggtgtctcg   ≈4863
atc                                                      ≈4866
```

FIG 16(vi)

EXON III CACTGCTTTGAAAC**gt**gagtgggggtgcgaacggag
gggtgcggggacgggcaggaacagggctggagggagtgccaccga
actttacctctggtctgatgccagacttgggcgtgaaagttgtgc
gtggatgcggcctggtgttctcctgagccccaggctgtgctgcag
ccggttacacccactccagttccctttgggtctcctggagggaac
cctgttcaggttattccagaatgttcttccagaacatttccacac
acttttgggtattctctccctttttctttcaacccaaagttcacc
actgaccatcccaccctcatccccccctcctggtggacggtgcggt
acagtgtggggcactgagccaaggccagcacccccgggccgctgt

...............INTRON C (716 BP)..............

gtggactccatcctgccaatcccacattggcgtggtgcatctccc
cattcctccttgggctgcatggggtgcccctggaggccttggct
caatgcaaggctccttgggacagctctgggaggtgacaagacccc
acccttctgctgcaggagcaggtcctagactttggttgtggtctg
tctgggctccttcatttctgcaggggaccctgggtgttagcaagt
agcagcaacaccacagtttcccctcctgcactggaccccagttgt
gctcaggtagccagccctccatccaggcccctgactgctctctt
ctcttctgcc**ag**ctat**ag**TGACCTTAGTGATCCC EXON IV

ALTERNATIVE
SPLICING EVENTS

SHORT ISOFORM

CACTGCTTTGAAACTGACCTTAGTGATCCC

H C F E T D L S D P

LONG ISOFORM

CACTGCTTTGAAAC**CTATAG**TGACCTTAGTGATCCC

H C F E T **Y** **S** D L S D P

FIGURE 17

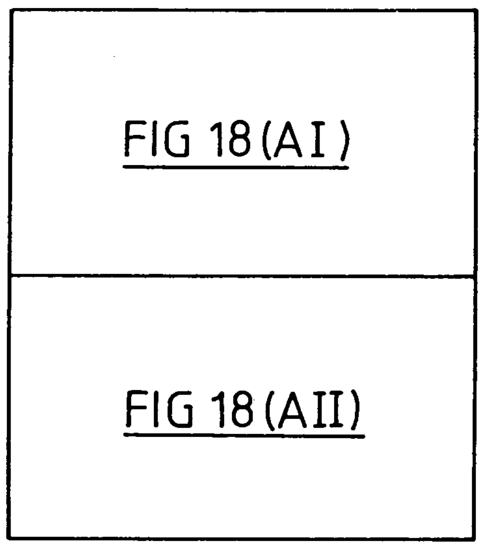


FIG 18(A)

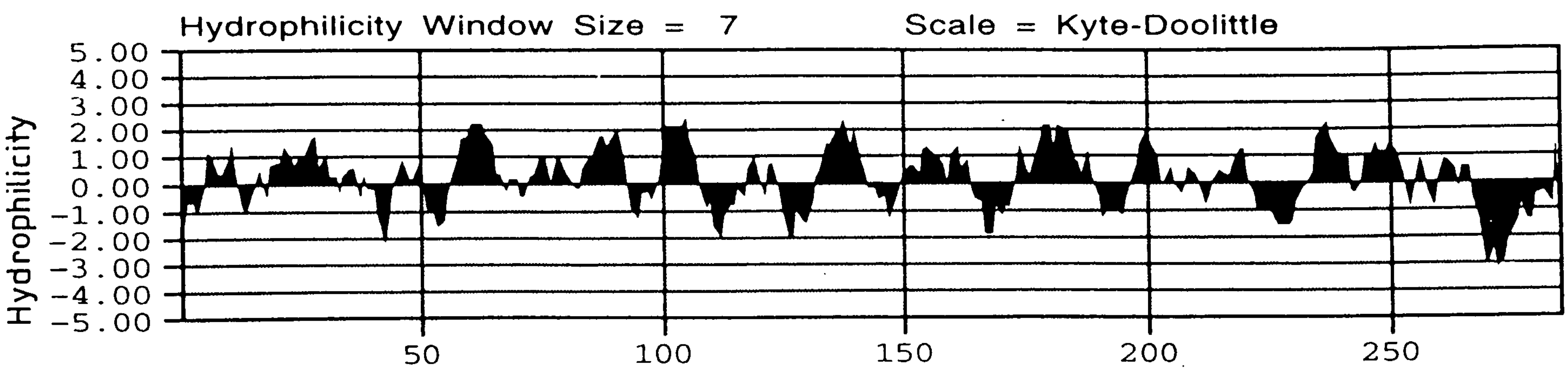
FIGURE 18 (AI)

```
1   CGACCTATTGTCAGGGCCCTGCGGTCACAGGACCATCCCTTCCCGTATAGTGGGTGGCGA
     D  L  L  S  G  P  C  G  H  R  T  I  P  S  R  I  V  G  G  D  20

61  TGATGCTGAGCTTGGCCGCTGGCCGTGGCAAGGGAGCCTGCGTGTATGGGGCAACCACTT
     D  A  E  L  G  R  W  P  W  Q  G  S  L  R  V  W  G  N  H  L  40

121 ATGTGGCGCAACCTTGCTCAACCGCCGCTGGGTGCTTACAGCTGCCCACTGCTTCCAAAA
     C  G  A  T  L  L  N  R  R  W  V  L  T  A  A  H  C  F  Q  K  60

181 GGATAACGATCCTTTTGACTGGACAGTCCAGTTTGGTGAGCTGACTTCCAGGCCATCTCT
     D  N  D  P  F  D  W  T  V  Q  F  G  E  L  T  S  R  P  S  L  80

241 CTGGAACCTACAGGCCTATTCCAACCGTTACCAAATAGAAGATATTTTCCTGAGCCCCAA
     W  N  L  Q  A  Y  S  N  R  Y  Q  I  E  D  I  F  L  S  P  K 100

301 GTACTCGGAGCAGTATCCCAATGACATAGCCCTGCTGAAGCTGTCATCTCCAGTCACCTA
     Y  S  E  Q  Y  P  N  D  I  A  L  L  K  L  S  S  P  V  T  Y 120

361 CAATAACTTCATCCAGCCCATCTGCCTCCTGAACTCCACGTACAAGTTTGAGAACCGAAC
     N  N  F  I  Q  P  I  C  L  L  N  S  T  Y  K  F  E  N  R  T 140

421 TGACTGCTGGGTGACCGGCTGGGGGCTATTGGAGAAGATGAGAGTCTGCCATCTCCCAA
     D  C  W  V  T  G  W  G  A  I  G  E  D  E  S  L  P  S  P  N 160
```

FIGURE 18 (AII)

```
481 CACTCTCCAGGAAGTGCAGGTAGCTATTATCAACAACAGCATGTGTAACCATATGTACAA
      T  L  Q  E  V  Q  V  A  I  I  N  N  S  M  C  N  H  M  Y  K 180

541 AAAGCCAGACTTCCGCACGAACATCTGGGGAGACATGGTTTGCGCTGGCACTCCTGAAGG
      K  P  D  F  R  T  N  I  W  G  D  M  V  C  A  G  T  P  E  G 200

601 TGGCAAGGATGCCTGCTTTGGTGACTCGGGAGGACCCTTGGCCTGCGACCAGGATACGGT
      G  K  D  A  C  F  G  D  S  G  G  P  L  A  C  D  Q  D  T  V 220

661 GTGGTATCAGGTTGGAGTTGTGAGCTGGGGAATAGGCTGTGGTCGCCCCAATCGCCCTGG
      W  Y  Q  V  G  V  V  S  W  G  I  G  C  G  R  P  N  R  P  G 240

721 AGTCTATACCAACATCAGTCATCACTACAACTGGATCCAGTCAACCATGATCCGCAATGG
      V  Y  T  N  I  S  H  H  Y  N  W  I  Q  S  T  M  I  R  N  G 260

781 GCTGCTCAGGCCTGACCCAGTCCCCTTGCTACTGTTTCTTACTCTGGCCTGGGCTTCCTC
      L  L  R  P  D  P  V  P  L  L  L  F  L  T  L  A  W  A  S  S 280
841 TTTGCTGAGGCCTGCCTGAGCCCACACGTGTACGTCACACCTGTGAGGTCAGGGTGTGTC
      L  L  R  P  A                                              285

901 TCTTTTGTATCTTGCTTGCTAATAAACCTGTTAATATTTAAAAAAAAAAAAAAAAAAA
```

FIG 18B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2.00
1.00
0.00
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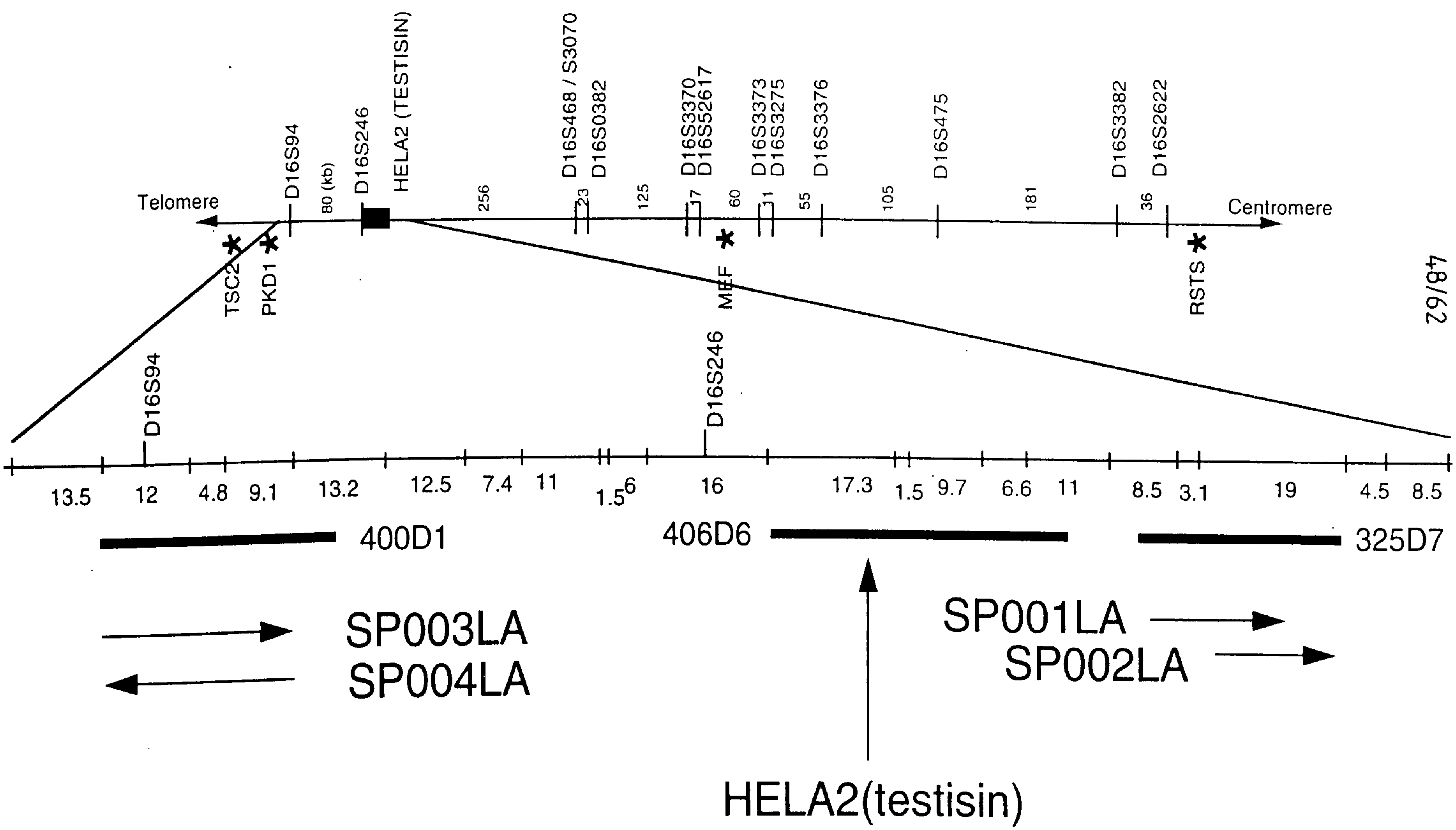
-2.00
-3.00
-4.00
-5.00
50
100
150
200
250

FIGURE 19


Telomere
Centromere
D16S94
80 (kb)
D16S246
HELA2 (TESTISIN)
256
D16S468 / S3070
D16S0382
23
125
D16S3370
D16S52617
17
60
D16S3373
D16S3275
11
55
D16S3376
105
D16S475
181
D16S3382
36
D16S2622
TSC2
PKD1
MEF
RSTS
D16S94
D16S246
13.5
12
4.8
9.1
13.2
12.5
7.4
11
1.5
6
16
17.3
1.5
9.7
6.6
11
8.5
3.1
19
4.5
8.5
400D1
406D6
325D7
SP003LA
SP004LA
SP001LA
SP002LA
HELA2(testisin)

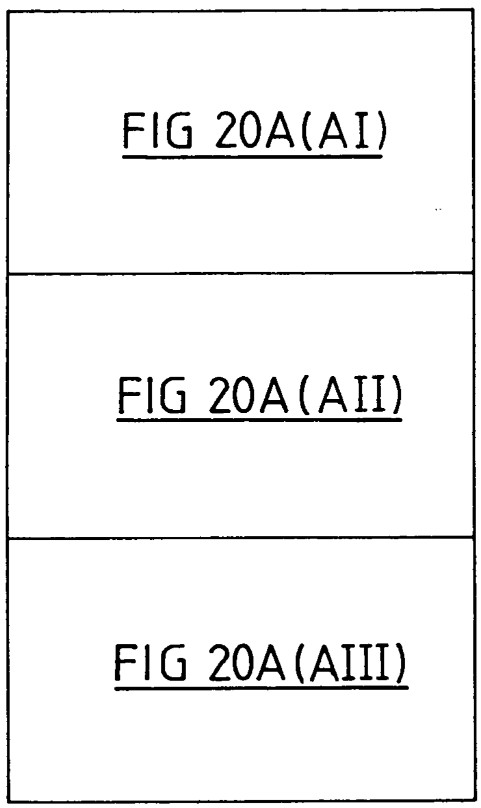


FIG 20A(A)

FIGURE 20A (AI)

```
    CTGAACCGGGTTGTGGCGGCGAGGACAGCACTGACAGCGAGTGGCCCTGGATCGTGAGC   60
1    L  N  R▽V  V  G  G  E  D  S  T  D  S  E  W  P  W  I  V  S

    ATCCAGAAGAATGGGACCCACCACTGCGCAGGTTCTCTGCTCACCAGCCGCTGGGTGATC  120
21   I  Q  K  N  G  T  H  H  C  A  G  S  L  L  T  S  R  W  V  I

    ACTGCTGCCCACTGTTTCAAGGACAACCTGAACAAACCATACCTGTTCTCTGTGCTGCTG  180
41   T  A  A  H  C  F  K  D  N  L  N  K  P  Y  L  F  S  V  L  L

    GGGGCCTGGCAGCTGGGGAACCCTGGCTCTCGGTCCCAGAAGGTGGGTGTTGCCTGGGTG  240
61   G  A  W  Q  L  G  N  P  G  S  R  S  Q  K  V  G  V  A  W  V

    GAGCCCCACCCTGTGTATTCCTGGAAGGAAGGTGCCTGTGCAGACATTGCCCTGGTGCGT  300
81   E  P  H  P  V  Y  S  W  K  E  G  A  C  A  D  I  A  L  V  R

    CTCGAGCGCTCCATACAGTTCTCAGAGCGGGTCCTGCCCATCTGCCTACCTGATGCCTCT  360
101  L  E  R  S  I  Q  F  S  E  R  V  L  P  I  C  L  P  D  A  S

    ATCCACCTCCCTCCAAACACCCACTGCTGGATCTCAGGCTGGGGAGCATCCAAGATGGA   420
121  I  H  L  P  P  N  T  H  C  W  I  S  G  W  G  S  I  Q  D  G
```

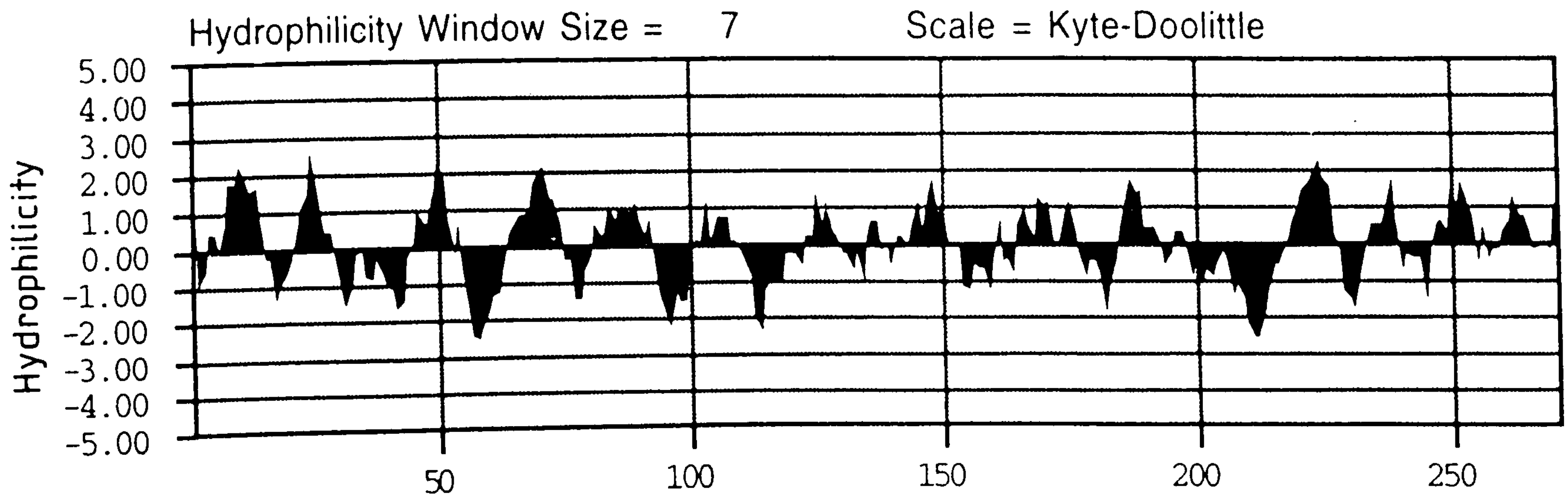
FIGURE 20A (AII)

```
     GTTCCCTTGCCCCACCCTCAGACCCTGCAGAAGCTGAAGGTTCCTATCATCGACTCGGGAA 480
141   V  P  L  P  H  P  Q  T  L  Q  K  L  K  V  P  I  I  D  S  E

     GTCTGCAGCCATCTGTACTGGCGGGGAGCAGGACAGGGACCCATCACTGAGGACATGCTG  540
161   V  C  S  H  L  Y  W  R  G  A  G  Q  G  P  I  T  E  D  M  L

     TGTGCCGGCTAACTTGGAGGGGGAGCGGGATGCTTGTCTGGGCGACTCCGGGGGCCCCCTC 600
181   C  A  G  Y  L  E  G  E  R  D  A  C  L  G  D  S  G  G  P  L

     ATGTGCCAGGTGGACGGCGCCTGGCTGCTGGCCGGCATCATCAGCTGGGGCGAGGGCTGT  660
201   M  C  Q  V  D  G  A  W  L  L  A  G  I  I  S  W  G  E  G  C

     GCCGAGCGCAACAGGCCCGGGGTCTACATCAGCCTCTCTGCGCACCGCTCCTGGGTGGAG  720
221   A  E  R  N  R  P  G  V  Y  I  S  L  S  A  H  R  S  W  V  E

     AAGATCGTGCAAGGGGTGCAGCTCCGCGGGCGCGCTCAGGGGGGTGGGGCCCTCAGGGCA  780
241   K  I  V  Q  G  V  Q  L  R  G  R  A  Q  G  G  G  A  L  R  A

     CCGAGCCAGGGCTCTGGGGCCGCCGCGCTCCTAGGGGCCCAGCGGGACGCGGGGCTCGG 840
261   P  S  Q  G  S  G  A  A  A  R  S

     ATCTGAAAGGCGGCCAGATCCACATCTGGATCTGGATCTGCGGCGGCCTCGGGCGGTTTC  900
     CCCCGCCGTAAATAGGCTCATCTACCTCTACCTCTGGGGGCCCGGACGGCTGCTGCGGAA  960
```

FIGURE 20A (AIII)

AGGAAACCCCCTCCCCGACCCGCCCGACGGCCTCAGGCCCCGCCTCCAAGGCATCAGGCC 1020
CCGCCCCAACGGCCTCATGTCCCCGCCCCCACGACTTCCGGCCCCGCCCCGGGCCCCAGCG 1080
CTTTTGTGTATATAAATGTTAATGATTTTTATAGGTATTTGTAACCCTGCCACATATCT 1140
TATTTATTCCTCCAATTTCAATAAA

FIG 20A (B)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Scale = Kyte-Doolittle
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3.00
2.00
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-3.00
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-5.00
50
100
150
200
250

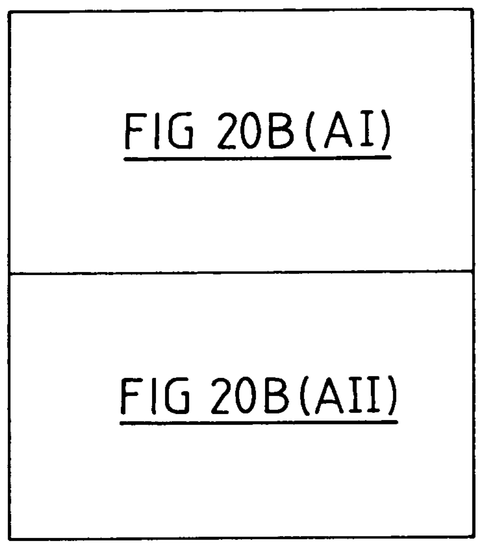


FIG 20B(A)

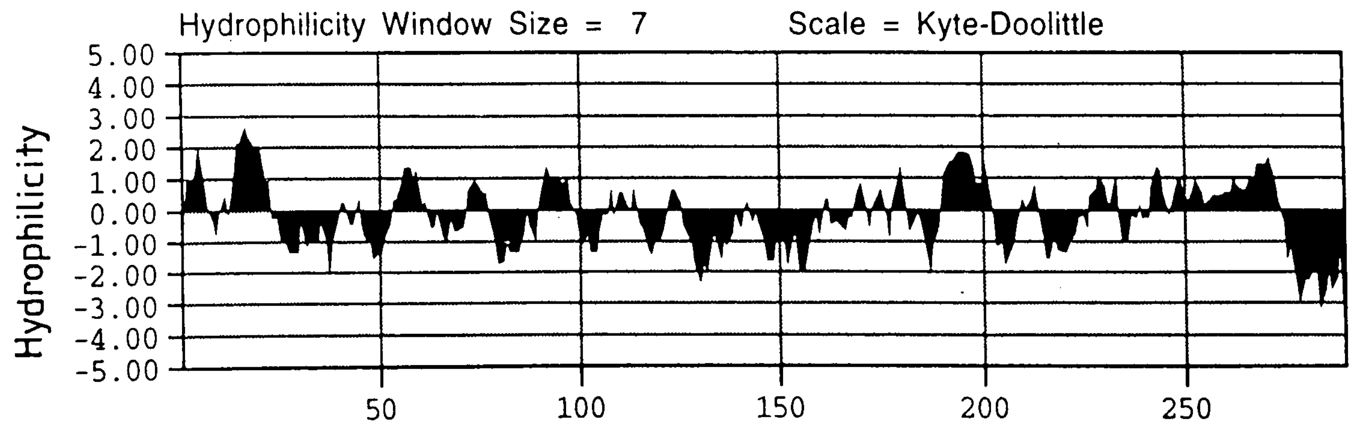
FIGURE 20B (AI)

```
     AATGCGGCCACTCCAAGGAGGCCGGGAGGATTGTGGGAGGCCAAGACACCCAGGAAGGAC  60
1      C  G  H  S  K  E  A  G  R▽I  V  G  G  Q  D  T  Q  E  G

     GCTGGCCGTGGCAGGTTGGCCTGTGGTTGACCTCAGTGGGGCATGTATGTGGGGGCTCCC 120
21   R  W  P  W  Q  V  G  L  W  L  T  S  V  G  H  V  C  G  G  S

     TCATCCACCCACGCTGGGTGCTCACAGCCGCCCACTGCTTCCTGAGGTCTGAGGATCCCG 180
41   L  I  H  P  R  W  V  L  T  A  A  H  C  F  L  R  S  E  D  P

     GGCTCTACCATGTTAAAGTCGGAGGGCTGACACCCTCACTTTCAGAGCCCCACTCGGCCT 240
61   G  L  Y  H  V  K  V  G  G  L  T  P  S  L  S  E  P  H  S  A

     TGGTGGCTGTGAGGAGGCTCCTGGTCCACTCCTCATACCATGGGACCACCACCAGCGGGG 300
81   L  V  A  V  R  R  L  L  V  H  S  S  Y  H  G  T  T  T  S  G

     ACATTGCCCTGATGGAGCTGGACTCCCCCTTGCAGGCCTCCCAGTTCAGCCCCATCTGCC 360
101  D  I  A  L  M  E  L  D  S  P  L  Q  A  S  Q  F  S  P  I  C

     TCCCAGGACCCCAGACCCCCCTCGCCATTGGGACCGTGTGCTGGGTAAACGGGCTGGGGG 420
121  L  P  G  P  Q  T  P  L  A  I  G  T  V  C  W  V  N  G  L  G

     TCCACTCAGGAGAGGCCCTGGCGAGTGTCCTTCAGGAGGTGGCTGTGCCCCTCCTGGACT 480
141  V  H  S  G  E  A  L  A  S  V  L  Q  E  V  A  V  P  L  L  D
```

FIGURE 20B (AII)

```
    CGAACATGTGTGAGCTGATGTACCACCTAGGAGAGCCCAGCCTGGCTGGCCAGCGCCTCA 540
161 S  N  M  C  E  L  M  Y  H  L  G  E  P  S  L  A  G  Q  R  L

    TCCAGGACGACATGCTCTGTGCTGGCTCTGTCCAGGGCAAGAAAGACTCCTGCCAGGGTG 600
181 I  Q  D  D  M  L  C  A  G  S  V  Q  G  K  K  D  S  C  Q  G

    ACTCCGGGGGGCCGCTGGTCTGCCCCATCAATGATACGTGGATCCAGGCCGGCATTGTGA 660
201 D  S  G  G  P  L  V  C  P  I  N  D  T  W  I  Q  A  G  I  V

    GCTGGGGATTCGGCTGTGCCCGGCCTTTCCGGCCTGGTGTCTACACCCAGGTGCTAAGCT 720
221 S  W  G  F  G  C  A  R  P  F  R  P  G  V  Y  T  Q  V  L  S

    ACACAGACTGGATTCAGAGAACCCTGGCTGAATCTCACTCAGGCATGTCTGGGGCCCGCC 780
241 Y  T  D  W  I  Q  R  T  L  A  E  S  H  S  G  M  S  G  A  R

    CAGGTGCCCCAGGATCCCACTCAGGCACCTCCAGATCCCACCCAGTGCTGCTGCTTGAGC 840
261 P  G  A  P  G  S  H  S  G  T  S  R  S  H  P  V  L  L  L  E

    TGTTGACCGTATGCTTGCTTGGGTCCCTGTGAACCATGAGCCATGGAGTCCGGGATCCCC 900
281 L  L  T  V  C  L  L  G  S  L

    TTTCTGGTAGGATTGATGGAATCTAATAATAAA
```

FIG 20B (B)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Hydrophilicity
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4.00
3.00
2.00
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100
150
200
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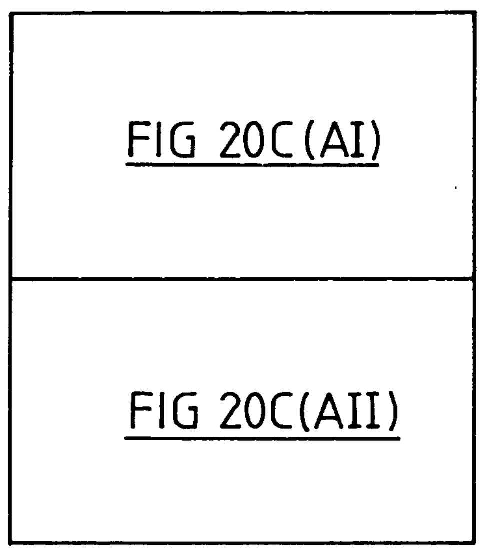


FIG 20C(A)

FIGURE 20C (AI)

CCTGTGGTCGCCCCAGGATGCTGAACCGAATGGTGGGCGGGCAGGACACGCAGGAGGGCG 60
1 $\boxed{C}$ G R P R M L N R▽M V G G Q D T Q E G

AGTGGCCCTGGCAAGTCAGCATCCAGCGCAACGGAAGCCACTTCTGCGGGGGCAGCCTCA 120
21 E W P W Q V S I Q R N G S H F $\boxed{C}$ G G S L

TCGCGGAGCAGTGGGTCCTGACGGCTGCGCACTGCTTCCGCAACACCTCTGAGACGTCCC 180
41 I A E Q W V L T A A H $\boxed{C}$ F R N T S E T S

TGTACCAGGTCCTGCTGGGGGCAAGGCAGCTAGTGCAGCCGGGACCACACGCTATGTATG 240
61 L Y Q V L L G A R Q L V Q P G P H A M Y

CCCGGGTGAGGCAGGTGGAGAGCAACCCCCTGTACCAGGGCACGGCCTCCAGCGCTGACG 300
81 A R V R Q V E S N P L Y Q G T A S S A D

TGGCCCTGGTGGAGCTGGAGGCACCAGTGCCCTTCACCAATTACATCCTCCCCGTGTGCC 360
101 V A L V E L E A P V P F T N Y I L P V $\boxed{C}$

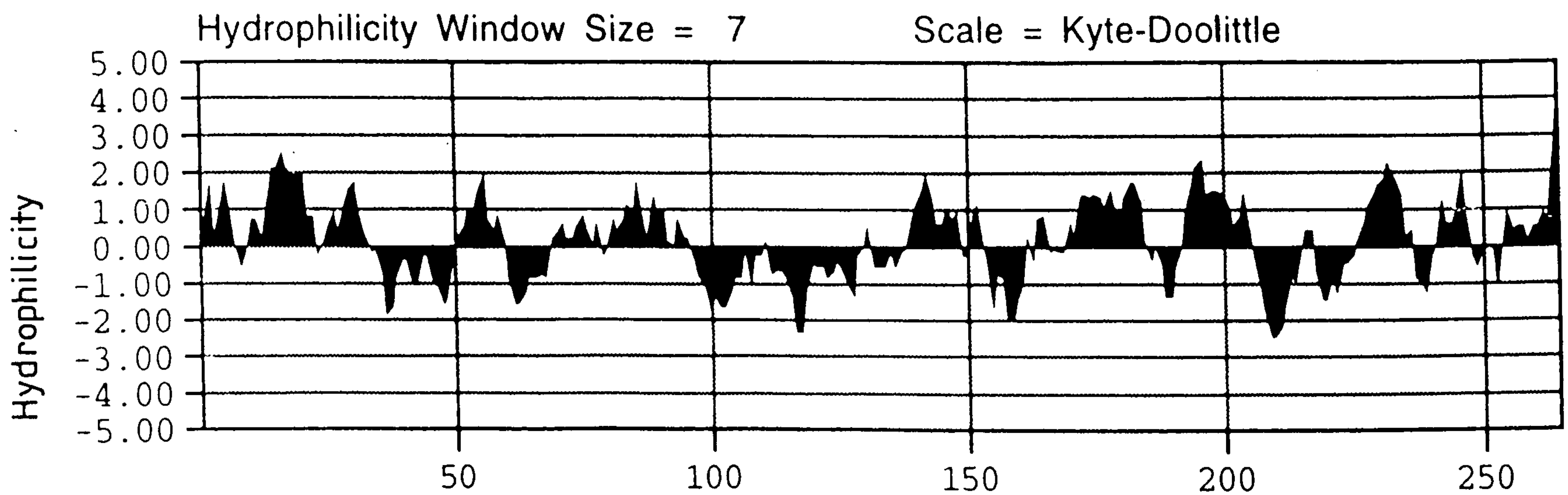
TGCCTGACCCCTCGGTGATCTTTGAGACGGGCATGAACTGCTGGGTCACTGGCTGGGGCA 420
121 L P D P S V I F E T G M N $\boxed{C}$ W V T G W G

GCCCCAGTGAGGAAGACCTCCTGCCCGAACCGCGGATCCTGCAGAAACTCGCTGTGCCCA 480
141 S P S E E D L L P E P R I L Q K L A V P

FIGURE 20C (AII)

```
    TCATCGACACACCCAAGTGCAACCTGCTCTACAGCAAAGACACCGAGTTTGGCTACCAAC 540
161 I  I  D  T  P  K  C  N  L  L  Y  S  K  D  T  E  F  G  Y  Q

    CCAAAACCATCAAGAATGACATGCTGTGCGCCGGCTTCGAGGAGGGCAAGAAGGATGCCT 600
181 P  K  T  I  K  N  D  M  L  C  A  G  F  E  E  G  K  K  D  A

    GCAAGGGCGACTCGGGCGGCCCCCTGGTGTGCCTCGTGGGTCAGTCGTGGCTGCAGGCGG 660
201 C  K  G  D  S  G  G  P  L  V  C  L  V  G  Q  S  W  L  Q  A

    GGGTGATCAGCTGGGGTGAGGGCTGTGCCCGCCAGAACCGCCCAGGTGTCTACATCCGTG 720
221 G  V  I  S  W  G  E  G  C  A  R  Q  N  R  P  G  V  Y  I  R

    TCACCGCCCACCACAACTGGATCCATCGGATCATCCCCAAACTGCAGTTCCAGCCAGCGA 780
241 V  T  A  H  H  N  W  I  H  R  I  I  P  K  L  Q  F  Q  P  A

    GGTTGGGCGGCCAGAAGTGAGACCCCCGGGGCCAGGAGCCCCTTGAGCAGAGCTCTGCAC 840
261 R  L  G  G  Q  K  *  D  P  R  G  Q  E  P  L  E  Q  S  S  A

    CCAGCCTGCCCGCCCACACCATCCTGCTGGTCCTCCCAGCGCTGCTGTTGCACCTGTGAG 900
281 P  S  L  P  A  H  T  I  L  L  V  L  P  A  L  L  L  H  L

    CCCCACCAGACTCATTTGTAAATAGCGCTCCTTCCTCCCCTCTCAAATACCCTTATTTTA 960
    TTTATGTTTCTCCCAATAAA
```

FIG 20C(B)


Hydrophilicity Window Size = 7
Scale = Kyte-Doolittle
5.00
4.00
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
-4.00
-5.00
Hydrophilicity
50
100
150
200
250

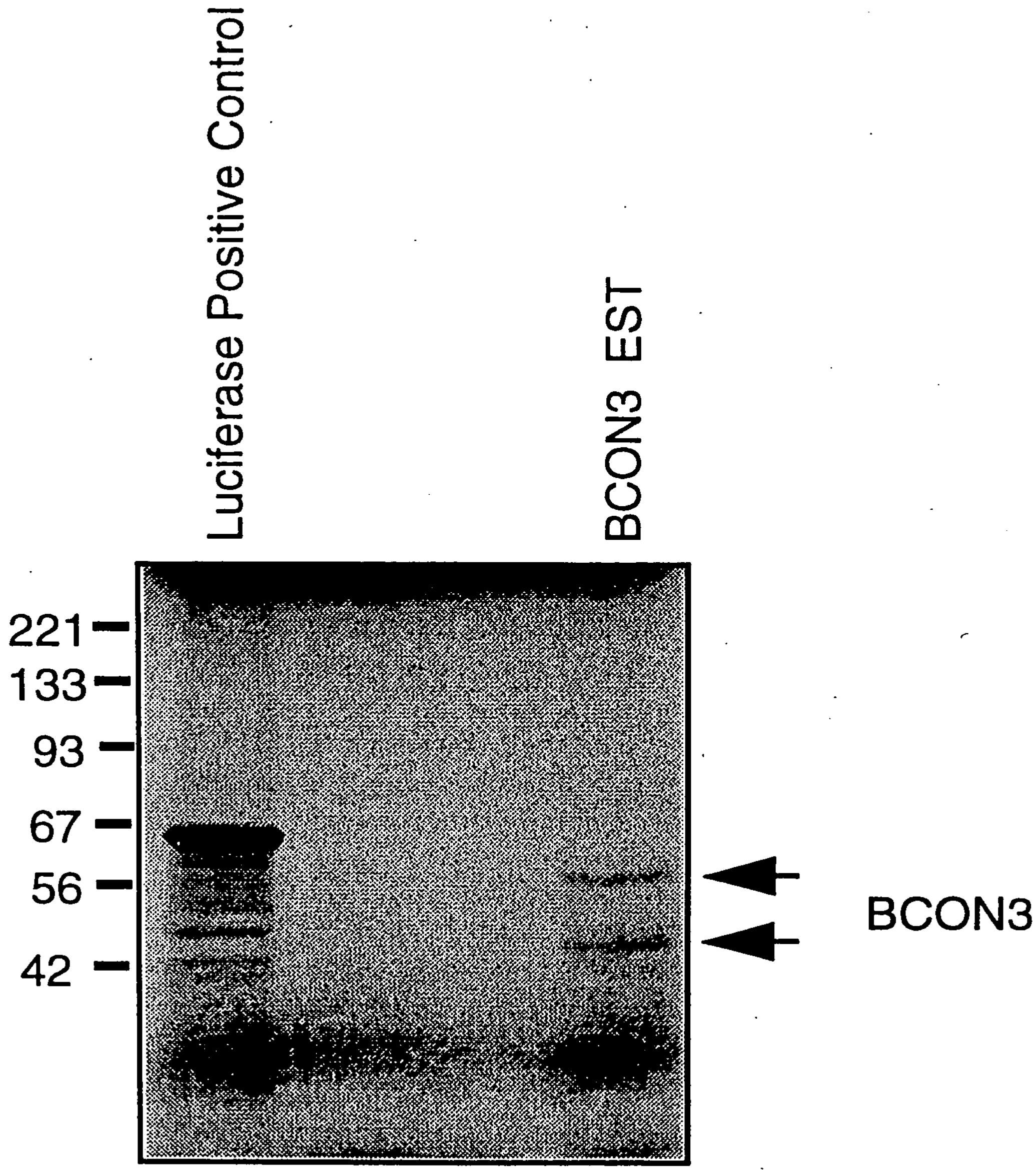

Luciferase Positive Control
BCON3 EST
221
133
93
67
56
42
BCON3

FIG 21